

IAP11 Rec'd PCT/PTO 10 AUG 2006

**APPARATUS AND METHOD FOR IDENTIFYING PEAKS IN
LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY DATA
AND FOR FORMING SPECTRA AND CHROMATOGRAMS**

[0001] The present invention claims the benefit of priority of U.S. Provisional Application 60/543,940, filed February 13, 2004, which is hereby incorporated by reference in its entirety.

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BACKGROUND

FIELD OF THE INVENTION

[0003] The present invention relates generally to liquid chromatography and mass spectrometry. More particularly, the present invention relates to detection and quantification of ions from data collected by an LC/MS system and subsequent or real-time analysis of such data.

BACKGROUND OF THE INVENTION

[0004] Mass spectrometers (MS) are well-known scientific instruments used widely for identifying and quantifying molecular species in a sample. During analysis, molecules from the sample are ionized to form ions that are introduced into the mass spectrometer for analysis. The mass spectrometer measures the mass-to-charge ratio (m/z) and intensity of the introduced ions.

[0005] Mass spectrometers are limited in terms of the number of ions they can reliably detect and quantify within a single spectrum. As a result, samples containing many molecular species may produce spectra that are too complex for interpretation or analysis using conventional mass spectrometers.

[0006] In addition, the concentration of molecular species can vary over a wide range. In biological samples, for example, there are typically a greater number of molecular species at lower concentrations than at higher concentrations. Thus, a significant fraction of ions appear at low

concentration. The low concentration is typically near the detection limit of the mass spectrometer. Moreover, at low concentration, ion detection also suffers from the presence of background noise and/or the presence of interfering background molecules. Consequently, detecting such low abundance species can be improved by removing as much of the background noise as possible and reducing the number of interfering species that are present in the spectrum at any one time.

[0007] A common technique used to reduce the complexity of such spectra is to perform a chromatographic separation prior to injecting the sample into the mass spectrometer. For example, peptides or proteins often produce clusters of ions that elute at a common time and that overlap in spectra. Separating the clusters from the different molecules in time simplifies interpretation of the spectra produced by such clusters.

[0008] Instruments commonly used to carry out such chromatographic separation include gas chromatographs (GCs) or liquid chromatographs (LCs). When coupled to a mass spectrometer, the resulting systems are referred to as GC/MS or LC/MS systems respectively. GC/MS or LC/MS systems are typically on-line systems in which the output of the GC or LC is coupled directly to the MS.

[0009] A combined LC/MS system provides an analyst with a powerful means to identify and to quantify molecular species in a wide variety of samples. Typical samples can contain a mixture of a few or thousands of molecular species. The molecules themselves can exhibit a wide range of properties and characteristics. For example, each molecular species can produce more than one ion. This can be seen in peptides where the mass of the peptide depends on the isotopic forms of its nuclei; and in the families of charge states into which an electrospray interface can ionize peptides and proteins.

[0010] In an LC/MS system, a sample is injected into the liquid chromatograph at a particular time. The liquid chromatograph causes the sample to elute over time resulting in an eluent that exits the liquid chromatograph. The eluent exiting the liquid chromatograph is continuously introduced into the ionization source of the mass spectrometer.

As the separation progresses, the composition of the mass spectrum generated by the MS evolves and reflects the changing composition of the eluent.

[0011] At regularly spaced time intervals, a computer-based system samples and records the spectrum on a storage device, such as a hard-disk drive. In conventional systems, these acquired spectra are analyzed after completion of the LC separation.

[0012] After acquisition, conventional LC/MS systems generate one-dimensional spectra and chromatograms. The response (or intensity) of an ion is the height or area of the peak as seen in either the spectrum or the chromatogram. To analyze spectra or chromatograms generated by conventional LC/MS systems, peaks in such spectra or chromatograms that correspond to ions must be located or detected. The detected peaks are analyzed to determine properties of the ions giving rise to the peaks. These properties include retention time, mass-to-charge ratio and intensity. Mass or mass-to-charge ratio (m/z) estimates for an ion are derived through examination of a spectrum that contains the ion. Retention time estimates for an ion are derived by examination of a chromatogram that contains the ion. The time location of a peak apex in a single mass-channel chromatogram provides an ion's retention time. The m/z location of a peak apex in a single spectral scan provides the ion's m/z value.

[0013] A conventional technique for detecting ions using an LC/MS system is to form a total ion chromatogram (TIC). Typically, this technique is applied if there are relatively few ions requiring detection. A TIC is generated by summing, within each spectral scan, all responses collected over all m/z values and plotting the sums against scan time. Ideally, each peak in a TIC corresponds to a single ion.

[0014] One problem with this method of detecting peaks in a TIC is possible co-elution of peaks from multiple molecules. As a result of such co-elution, each isolated peak seen in the TIC may not correspond to a unique ion. A conventional method for isolating such co-eluted peaks is to select the apex of one peak from the TIC and collect spectra for the time corresponding to the selected peak's apex. The resulting spectral plot is a

series of mass peaks, each presumably corresponding to a single ion eluting at a common retention time.

[0015] For complex mixtures, co-elution also typically limits summing of spectral responses to sums only over a subset of collected channels, *e.g.*, by summing over a restricted range of m/z channels. The summed chromatogram provides information about ions detected within the restricted m/z range. In addition, spectra can be obtained for each chromatographic peak apex. To identify all ions in this manner, multiple summed chromatograms are generally required.

[0016] Another difficulty encountered with peak detection is detector noise. A common technique for mitigating detector noise effects is to signal-average spectra or chromatograms. For example, the spectra corresponding to a particular chromatographic peak can be co-added to reduce noise effects. Mass-to-charge ratio values as well as peak areas and heights can be obtained from analyzing the peaks in the averaged spectrum. Similarly, co-adding chromatograms centered on the apex of a spectral peak can mitigate noise effects in chromatograms and provide more accurate estimates of retention time as well as chromatographic peak areas and heights.

[0017] Aside from these problems, additional difficulties are encountered when conventional peak detection algorithms are used to detect chromatographic or spectral peaks. If performed manually, such conventional methods are not only subjective, but are also quite tedious. Even when performed automatically, such methods can be subjective due to, for example, the subjective choices for thresholds to use to identify peaks. Further, these conventional methods tend to be inaccurate because they analyze data using only a single extracted spectrum or chromatogram, and do not provide ion parameter estimates having the highest statistical precision or lowest statistical variance. Finally, conventional peak-detection techniques do not necessarily provide uniform, reproducible results for ions at low concentration, or for complex chromatograms, where co-elution and ion interference tend to be common problems.

BRIEF SUMMARY OF THE INVENTION

[0018] One aspect of embodiments of the present invention is to detect the ions measured in the spectral scans collected in an LC/MS system and to determine from these scans the retention time, mass-to-charge ratio, and intensity of each ion. Ion parameters, such as mass-to-charge ratio (m/z), retention time and intensity are accurately and optimally estimated by creating a data matrix and convolving the data matrix with a fast, linear, two-dimensional finite impulse response (FIR) filter to generate an output convolved matrix. A peak detection algorithm is applied to the output convolved matrix to identify peaks corresponding to ions in the sample. By analyzing the peaks detected in the filtered matrix, ion parameters such as retention time, mass-to-charge ratio (m/z) and intensity can be estimated and recorded. In addition, other peak parameters such as full width at half maximum (FWHM) in both the spectral and chromatographic directions can be estimated and stored.

[0019] While providing a substantially complete accounting of the ions detected by an LC/MS apparatus, embodiments of the present invention also reduces the effects of noise and help resolve partially co-eluted compounds and unresolved ions that are typically observed in conventional LC/MS outputs. Optimal estimation of ion parameters increases the precision and reproducibility of the estimates.

[0020] Spectral and chromatographic complexity can be significantly reduced using embodiments of the present invention. For example, in embodiments of the present invention, a list or table of parameters associated with each detected ion is created and stored, rather than storing entire data sets associated with collected spectra or chromatographs.

[0021] Using the created ion parameter list, embodiments of the present invention extract subsets of ions that have desired properties or relationships. These subsets are used to create spectra and chromatograms that are less complex than those generated using conventional systems. For example, ions from a common parent molecule typically have essentially identical retention times in an LC/MS chromatogram. Embodiments of the present invention allow specification of a retention time window that can be

used to identify those ions that likely come from a common parent ion. In this manner, embodiments of the present invention facilitate identification and grouping of related ions while ignoring unrelated ions.

[0022] The reduced complexity spectra and reduced-complexity chromatograms that can be generated using embodiments of the present invention are a significant improvement over those obtained using conventional systems that simply extract a single spectrum (or an average of spectra) from the LC/MS data. This is because such conventionally generated spectra typically are contaminated by ions from the leading or tailing edge of peaks that are unrelated to the ions of interest.

[0023] The ions retained in the reduced complexity spectra and reduced-complexity chromatograms using embodiments of the present invention can be further analyzed by methods known in the prior art. For example, these methods include methods for determining the mass or identity of the common parent molecule.

[0024] Use of the present invention provides enhanced completeness, accuracy, and reproducibility of LC/MS experimental results by improving completeness, accuracy, and reproducibility of results obtained from a single injection. In addition, reduction in complexity further simplifies the interpretation of spectra and chromatograms due to the presence of fewer ions, reduction in noise background, and partial resolution of co-eluted compounds and interfering ions.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] Figure 1 is a schematic diagram of an exemplary LC/MS system according to an embodiment of the present invention.

[0026] Figure 2 is a diagram of an exemplary chromatographic or spectral peak.

[0027] Figure 3 illustrates exemplary spectra for three ions produced during an exemplary LC/MS experiment for three times.

[0028] Figure 4 illustrates chromatograms corresponding to the exemplary ions of Figure 3.

[0029] Figure 5 is a flow chart for a method for processing data according to an embodiment of the present invention.

- [0030] Figure 6 is a graphical flow chart for a method for processing data according to an embodiment of the present invention.
- [0031] Figure 7 is a graphical flow chart for a method for determining thresholds for use in detecting ions according to an embodiment of the present invention.
- [0032] Figure 8 illustrates an exemplary data matrix according to an embodiment of the present invention.
- [0033] Figure 9 illustrates a contour plot representation of an exemplary data matrix created from the data of Figures 3 and 4 according to an embodiment of the present invention.
- [0034] Figure 10 is a flow chart for a simplified method of processing data in the absence of noise according to an embodiment of the present invention.
- [0035] Figure 11 illustrates an effect of a co-eluting ion on the exemplary data matrix of Figure 9.
- [0036] Figure 12 illustrates a "shoulder" effect of a co-eluting ion on the exemplary data illustrated in Figure 3.
- [0037] Figure 13 illustrates how noise affects exemplary data in a data matrix created according to embodiments of the present invention.
- [0038] Figure 14A illustrates spectra for three ions corresponding to the exemplary data illustrated in the data matrix shown in Figure 13.
- [0039] Figure 14B illustrates chromatograms for three ions corresponding to the exemplary data illustrated in the data matrix shown in Figure 13.
- [0040] Figure 15 illustrates an exemplary one-dimensional apodized Savitzky-Golay second-derivative filter according to an embodiment of the present invention.
- [0041] Figure 16A illustrates the cross section of an exemplary one-dimensional filter in the spectral (m/z) direction according to an embodiment of the present invention.
- [0042] Figure 16B illustrates the cross section of an exemplary one-dimensional filter in the chromatographic (time) direction according to an embodiment of the present invention.

- [0043] Figure 16C illustrates the cross section of an exemplary one-dimensional smoothing filter f_1 in the spectral (m/z) direction according to an embodiment of the present invention.
- [0044] Figure 16D illustrates the cross section of an exemplary one-dimensional second derivative filter g_1 in the chromatographic direction according to an embodiment of the present invention.
- [0045] Figure 16E illustrates the cross section of an exemplary one-dimensional smoothing filter g_2 in the chromatographic direction according to an embodiment of the present invention.
- [0046] Figure 16F illustrates the cross section of an exemplary one-dimensional second-derivative filter f_2 in the spectral (m/z) direction according to an embodiment of the present invention.
- [0047] Figure 17A illustrates an exemplary peak that can be generated by LC/MS data as stored in a data matrix according to embodiments of the present invention.
- [0048] Figure 17B illustrates a point-source response (finite impulse response) of an exemplary rank-2 filter according to an embodiment of the present invention.
- [0049] Figure 17C illustrates a simulation of two LC/MS peaks having equal mass and that are nearly, but not identically, co-incident in time.
- [0050] Figure 17D illustrates the peak cross section in mass of the two-peak simulation of Figure 17C.
- [0051] Figure 17E illustrates the peak cross section in time of the two-peak simulation of Figure 17C.
- [0052] Figure 17F illustrates the effect of adding counting (shot) noise to the two-peak simulation of Figure 17C.
- [0053] Figure 17G illustrates the peak cross section in mass of the added noise two-peak simulation of Figure 17F.
- [0054] Figure 17H illustrates the peak cross section in time of the added noise two-peak simulation of Figure 17F.
- [0055] Figure 17I illustrates the result convolving a rank-2 filter with simulated data of Figure 17F.

- [0056] Figure 17J illustrates the peak cross section in mass of the result illustrated in Figure 17I.
- [0057] Figure 17K illustrates the peak cross section in time of the result illustrated in Figure 17I.
- [0058] Figure 18 illustrates a flow chart for performing real-time processing of data according to an embodiment of the present invention.
- [0059] Figure 19 is a graphical illustration of a method for performing real-time processing of a data according to the method of the flow chart of Figure 18.
- [0060] Figure 20 is a flow chart for a method for determining appropriate thresholds according to an embodiment of the present invention.
- [0061] Figure 21 is a flow chart for a method for determining a peak purity metric according to an embodiment of the present invention.
- [0062] Figure 22A illustrates an exemplary LC/MS data matrix resulting from two parent molecules and the resulting multiplicity of molecules.
- [0063] Figure 22B illustrates an exemplary complex spectrum corresponding to the data of Figure 22A at a time t1.
- [0064] Figure 22C illustrates an exemplary complex spectrum corresponding to the data of Figure 22A at a time t2.
- [0065] Figure 23 is a graphical chart illustrating how related ions can be identified in the unmodified and modified ion lists generated by an embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

- [0066] Embodiments of the present invention can be applied to a variety of applications including large-molecule, non-volatile analytes that can be dissolved in a solvent. Although embodiments of the present invention are described hereinafter with respect to LC or LC/MS systems, embodiments of the present invention can be configured for operation with other analysis techniques, including GC and GC/MS systems.
- [0067] Figure 1 is a schematic diagram of an exemplary LC/MS system 101 according to an embodiment of the present invention. LC/MS analysis is performed by automatically or manually injecting a sample 102 into a liquid chromatograph 104. A high pressure stream of chromatographic solvent

provided by pump 103 and injector 105 forces sample 102 to migrate through a chromatographic column 106 in liquid chromatograph 104. Column 106 typically comprises a packed column of silica beads whose surface comprises bonded molecules. Competitive interactions between the molecular species in the sample, the solvent and the beads determine the migration velocity of each molecular species.

[0068] A molecular species migrates through column 106 and emerges, or elutes, from column 106 at a characteristic time. This characteristic time commonly is referred to as the molecule's retention time. Once the molecule elutes from column 106, it can be conveyed to a detector, such as a mass spectrometer 108.

[0069] A retention time is a characteristic time. That is, a molecule that elutes from a column at retention time t in reality elutes over a period of time that is essentially centered at time t . The elution profile over the time period is referred to as a chromatographic peak. The elution profile of a chromatographic peak can be described by a bell-shaped curve. The peak's bell shape has a width that typically is described by its full width at half height, or half-maximum (FWHM). The molecule's retention time is the time of the apex of the peak's elution profile. Spectral peaks appearing in spectra generated by mass spectrometers have a similar shape and can be characterized in a similar manner. Figure 2 illustrates an exemplary chromatographic or spectral peak 202 having a peak apex 204. The FWHM and height of the peak 202 are also illustrated in Figure 2.

[0070] For purposes of subsequent description, peaks are assumed to have a Gaussian profile as shown in Figure 2. For a Gaussian profile, the FWHM is approximately 2.35 times the standard deviation σ of the Gaussian profile.

[0071] Chromatographic peak width is independent of peak height and is substantially a constant characteristic of a molecule for a given separation method. In the ideal case, for a given chromatographic method all molecular species would elute with the same peak width. However, typically peak widths change as a function of retention time. For example, molecules that elute at the end of a separation can display peak widths that

are several times wider than peak widths associated with molecules that elute early in the separation.

[0072] In addition to its width, a chromatographic or spectral peak has a height or area. Generally, the height and area of the peak are proportional to the amount or mass of the species injected into the liquid chromatograph. The term intensity commonly refers to either the height or area of the chromatographic or spectral peak.

[0073] Although chromatographic separation is a substantially continuous process, detectors analyzing the eluent typically sample the eluent at regularly spaced intervals. The rate at which a detector samples the eluent is referred to as the sample rate or sample frequency. Alternatively, the interval at which a detector samples the eluent is referred to as the sampling interval or sample period. Because the sample period must be long enough so that the system adequately samples the profile of each peak, the minimum sample period is limited by the chromatographic peak width. As an example, the sample period can be set so that approximately five (5) measurements are made during the FWHM of a chromatographic peak.

[0074] In an LC/MS system, the chromatographic eluent is introduced into a mass spectrometer (MS) 108 for analysis as shown in Figure 1. MS 108 comprises a desolvation system 110, an ionizer 112, a mass analyzer 114, a detector 116, and a computer 118. When the sample is introduced into MS 108, desolvation system 110 removes the solvent, and ionizing source 112 ionizes the analyte molecules. Ionization methods to ionize molecules that evolve from LC 104 include electron-impact (EI), electrospray (ES), and atmospheric chemical ionization (APCI). Note that in APCI, the order of ionization and desolvation is reversed.

[0075] The ionized molecules are then conveyed to mass analyzer 114. Mass analyzer 114 sorts or filters the molecules by their mass-to-charge ratio. Mass analyzers, such as mass analyzer 114 that are used to analyze ionized molecules in MS 108 include quadrupole mass analyzers (Q), time-of-flight (TOF) mass analyzers, and Fourier-transform-based mass spectrometers (FTMS).

[0076] Mass analyzers can be placed in tandem in a variety of configurations, including, *e.g.*, quadrupole time-of-flight (Q-TOF) mass analyzers. A tandem configuration enables on-line collision modification and analysis of an already mass-analyzed molecule. For example, in triple quadrupole based mass analyzers (such as Q1-Q2-Q3 or Q1-Q2-TOF mass analyzers), the second quadrupole (Q2), imports accelerating voltages to the ions separated by the first quadrupole (Q1). These ions, collide with a gas expressly introduced into Q2. The ions fragment as a result of these collisions. Those fragments are further analyzed by the third quadrupole (Q3) or by the TOF. Embodiments of the present invention are applicable to spectra and chromatograms obtained from any mode of mass-analysis such as those described above.

[0077] Molecules at each value for m/z are then detected with detection device 116. Exemplary ion detection devices include current measuring electrometers and single ion counting multi-channel plates (MCPs). The signal from an MCP can be analyzed by a discriminator followed by a time-domain-converter (TDC) or by an analog to digital (ATD) converter. For purposes of the present description, an MCP detection-based system is assumed. As a result, detector response is represented by a specific number of counts. This detector response (*i.e.*, number of counts) is proportional to the intensity of ions detected at each mass-to-charge-ratio interval.

[0078] An LC/MS system outputs a series of spectra or scans collected over time. A mass-to-charge spectrum is intensity plotted as a function of m/z . Each element, a single mass-to-charge ratio, of a spectrum is referred to as a channel. Viewing a single channel over time provides a chromatogram for the corresponding mass-to-charge ratio. The generated mass-to-charge spectra or scans can be acquired and recorded by computer 118 and stored in a storage medium such as a hard-disk drive that is accessible to computer 118. Typically, a spectrum or chromatogram is recorded as an array of values and stored by computer system 118. The array can be displayed and mathematically analyzed.

[0079] The specific functional elements that make up an MS system, such as MS 108, can vary between LC/MS systems. Embodiments of the present

invention can be adapted for use with any of a wide range of components that can make up an MS system.

[0080] After chromatographic separation and ion detection and recordation, the data is analyzed using a post-separation data analysis system (DAS). In an alternate embodiment of the present invention, the DAS performs analysis in real-time or near real-time. The DAS is generally implemented by computer software executing on a computer such as computer 118 shown in Figure 1. Computers that can be configured to execute the DAS as described herein are well known to those skilled in the art. The DAS is configured to perform a number of tasks, including providing visual displays of the spectra and/or chromatograms as well as providing tools for performing mathematical analysis on the data. The analyses provided by the DAS include analyzing the results obtained from a single injection and/or the results obtained from a set of injections to be viewed and further analyzed. Examples of analyses applied to a sample set include the production of calibration curves for analytes of interest, and the detection of novel compounds present in the unknowns, but not in the controls. A DAS according to embodiments of the present invention is described herein.

[0081] Figure 3 illustrates exemplary spectra for three ions (ion 1, ion 2 and ion 3) produced during an exemplary LC/MS experiment. Peaks associated with ion 1, ion 2 and ion 3 appear within a limited range of retention time and m/z . For the present example, it is assumed that the mass-to-charge ratios of ion 1, ion 2 and ion 3 are different, and that the molecular parents of the ions eluted at nearly, but not exactly, identical retention times. As a result, the elution profiles of the respective molecules overlap or co-elute. Under these assumptions, there is a time when all three molecules are present in the ionizing source of the MS. For example, the exemplary spectrum illustrated in Figure 3 were collected when all three ions were present in the MS ionization source. This is apparent because each spectrum exhibits a peak associated with each of ions 1, 2 and 3. As can be seen in the exemplary spectra illustrated in Figure 3, there is no overlap of spectral peaks. The lack of overlap indicates that the mass spectrometer

resolved these spectral peaks. The location of the apex of the peaks corresponding to each of ions 1, 2 and 3 represents its mass-to-charge ratio.

[0082] It is not possible to determine precise retention times or even relative retention times at which ions in a spectrum elute using only a single spectrum. For example, it can be seen that at the time the data for Spectrum B was collected, all three molecules associated with ions 1, 2 and 3 were eluting from the column. However, analyzing only Spectrum B, it is not possible to determine a relationship between the elution times of ions 1, 2 and 3. Thus, Spectrum B could have been collected at a time corresponding to the beginning of a chromatographic peak, as the molecule began to elute from the column, or from the end of the chromatographic peak, when the molecule was nearly finished eluting or at some time in between.

[0083] More accurate information related to retention time can be obtained by examining successive spectra. This additional information can include the retention time of the eluting molecules or at least the elution order. For example, assume Spectra A, B, and C shown in Figure 3 were collected successively such that Spectrum A was collected at time t_A ; Spectrum B was collected at a later time t_B ; and Spectrum C was collected at time t_C , which is a time later than time t_B . Then, the elution order of the respective molecules can be determined by examining the relative heights of the peaks appearing in spectra successively collected as time progresses from t_A to t_C . Such examination reveals that as time progresses ion 2 decreases in intensity relative to ion 1, and that ion 3 increases in intensity relative to ion 1. Therefore, ion 2 elutes before ion 1, and ion 3 elutes after ion 1.

[0084] This elution order can be verified by generating chromatograms corresponding to each peak found in a spectrum. This can be accomplished by obtaining the m/z value at the apex of each of the peaks corresponding to ions 1, 2 and 3. Given these three m/z values, the DAS extracts from each spectrum the intensity obtained at that m/z for each scan. The extracted intensities are then plotted versus elution time. Such a plot is illustrated in Figure 4. It can be seen that the plots in Figure 4 represent the chromatograms for ions 1, 2, and 3 at the m/z values obtained by examining the peaks in Figure 3. Each chromatogram contains a single peak.

Examination of the chromatograms for ions 1, 2 and 3 as illustrated in Figure 4 confirms that ion 2 elutes at the earliest time and that ion 3 elutes at the latest time. The apex location in each of the chromatograms shown in Figure 4 represents the elution time for the molecule corresponding to the respective ions.

[0085] With this introduction in mind, embodiments of the present invention relate to analyzing experimental analysis outputs, such as spectra and chromatograms, to optimally detect ions and quantify parameters related to the detected ions. Moreover, embodiments of the present invention can provide significantly simplified spectra and chromatograms.

[0086] Figure 5 is a flow chart 500 for processing experimental analysis output, such as spectra and chromatograms. Flow chart 500 can be embodied in a number of ways including in the DAS described above. In the embodiment of the present invention illustrated in Figure 5, analysis proceeds as follows:

STEP 502: Create a two-dimensional data matrix having chromatographic and spectral data.

STEP 504: Specify a two-dimensional convolution filter to apply to the data matrix.

STEP 506: Apply the two-dimensional convolution filter to the data matrix. For example, the data matrix can be convolved with the two-dimensional filter.

STEP 508: Detect peaks in the output of the application of the two-dimensional filter to the data matrix. Each detected peak is deemed to correspond to an ion. Thresholding can be used to optimize peak detection.

STEP 510: Extract ion parameters for each detected peak. The parameters include ion characteristics such as retention time, mass-to-charge ratio, intensity, peak width in the spectral direction and/or peak width in the chromatographic direction.

STEP 512: Store the ion parameters associated with extracted ions in a list or table. Storage can be performed as each peak is detected or after a plurality or all of the peaks have been detected.

STEP 514: Use the extracted ion parameters to post-process the data. For example, the ion parameter table can be used to simplify the data. Such simplification can be accomplished, for example, by windowing to reduce spectral or chromatographic complexity. Properties of the molecules can be inferred from the simplified data.

[0087] Figures 6 and 7 are graphical flow charts describing the foregoing steps of flow chart 500. Figure 6 is a graphical flowchart 602 of a method for processing LC/MS data according to an embodiment of the present invention. More particularly, each element of graphical flowchart 602 illustrates the result of a step according to an embodiment of the present invention. Element 604 is an exemplary LC/MS data matrix created according to an embodiment of the present invention. As described below, the LC/MS data matrix can be created by placing LC/MS spectra collected at successive times in successive columns of a data matrix. Element 606 is an exemplary two-dimensional convolution filter that can be specified according to desired filtering characteristics. Considerations for specifying the two-dimensional filter are described in more detail below. Element 608 represents application of the two-dimensional filter of element 606 to the LC/MS data matrix of element 604 according to an embodiment of the present invention. An exemplary such application of the two-dimensional filter to the LC/MS data matrix is a two-dimensional convolution wherein the LC/MS data matrix is convolved with the two-dimensional convolution filter. The output of the filtering step is the output data matrix, an example of which is illustrated as element 610. Where the application of the filter to the data matrix comprises a convolution, the output is an output convolved matrix.

[0088] Element 612 illustrates an exemplary result of performing peak detection on the output data matrix to identify or detect peaks associated with ions. Thresholding can be used to optimize the peak detection. At this point, the ions are considered detected. Element 614 is an exemplary list or table of the ion properties created using the detected ions.

[0089] Figure 7 is a graphical flowchart 702 illustrating results of determining a detection threshold and its application to further consolidate the ion parameter table according to an embodiment of the present invention. Element 706 represents exemplary peak data accessed from the ion parameter list, Element 704. Element 706 illustrate results of determining a detection threshold using the accessed data. The determined threshold is applied to the ion parameter list generated as Step 704 to generate an edited ion parameter list an emample of which is illustrated as Step 708. The foregoing steps are now explained in more detail.

Step 1: Create data matrix

[0090] Rather than view the output of an LC/MS analysis as distinct series of spectra and chromatograms, it is advantageous to configure the LC/MS output as a data matrix of intensities. In an embodiment of the present invention, the data matrix is constructed by placing the data associated with each successive spectrum collected at increasing time in successive columns of a data matrix thereby creating a two-dimensional data matrix of intensities. Figure 8 illustrates an exemplary such data matrix 800 in which five (5) spectra successively collected in time are stored in successive columns 801-805 of data matrix 800. When the spectra are stored in this manner, the rows of data matrix 800 represent chromatograms at corresponding m/z values in the stored spectra. These chromatograms are indicated by rows 811-815 in data matrix 800. Thus, in matrix form, each column of the data matrix represents a spectrum collected at a particular time, and each row represents a chromatogram collected at a fixed m/z. Each element of the data matrix is an intensity value collected at a particular time (in the corresponding chromatogram) for a particular m/z (in the corresponding spectrum). Although the present disclosure assumes column-oriented spectral data and row-oriented chromatographic data, in alternate embodiments of the present invention, the data matrix is oriented such that rows represent spectra and columns represent chromatograms.

[0091] Figure 9 is an exemplary graphical representation (in particular, a contour plot) of a data matrix generated as described above by storing spectral data in successive columns of the data matrix. In the contour plot

illustrated in Figure 9, each of ions 1, 2 and 3 appears as an island of intensity. The contour plot distinctly shows not only the presence of the three ions, but also that the elution order is ion 2, followed by ion 1, followed by ion 3. Figure 9 also shows three apices 902a, 902b and 902c. Apex 902a corresponds to ion 1, apex 902b corresponds to ion 2 and apex 902c corresponds to ion 3. The locations of apices 902a, 902b, and 902c correspond to the m/z and retention time for ions 1, 2 and 3 respectively. The height of the apex above the zero value floor of the contour plot is a measure of the ion's intensity. The counts or intensities associated with a single ion are contained within an ellipsoidal region or island. The FWHM of this region in the m/z (column) direction is the FWHM of the spectral (mass) peak. The FWHM of this region in the row (time) direction is the FWHM of the chromatographic peak.

[0092] The innermost of the concentric contours forming an island identifies the element having the highest intensity. This local maximum or maximal element has an intensity greater than its nearest neighbors. For example, for two-dimensional data contours, a local maximum or apex is any point whose amplitude is greater than its nearest-neighbor elements. In one embodiment of the present invention, a local maximum or apex must be greater than eight (8) nearest neighbor elements. For example in the Table 1, the central element is a local maximum because each of the 8 adjoining elements has a value less than 10.

8.5	9.2	6.8
9.2	10.0	8.4
7.9	8.5	7.2

Table 1: Example showing maximum

[0093] There are six lines drawn through the contour plot of Figure 9. The three horizontal lines, labeled ion 1, ion 2 and ion 3, identify cross sections corresponding to the chromatograms for ions 1, 2 and 3 respectively as illustrated in Figure 4. The three vertical lines, labeled A, B and C, identify cross sections corresponding to the mass spectra 3A, 3B and 3C respectively as illustrated in Figure 3.

[0094] After the data matrix is created, ions are detected. For each detected ion, ion parameters, such as retention time, m/z and intensity, are obtained. If the data matrix is free of noise and if the ions do not interfere with one another (e.g., by chromatographic co-elution and spectral interference), then each ion produces a unique, isolated island of intensity, as illustrated in the contour plot of Figure 9.

[0095] As shown in Figure 9, each island contains a single maximal element. Where there is no noise, co-elution or interference, ion detection and parameter quantification according to an embodiment of the present invention proceeds as follows as shown in flow chart 1000 in Figure 10:

STEP 1001: Form Data Matrix

STEP 1002: Interrogate each element in the data matrix.

STEP 1004: Identify all elements that are local maxima of intensity and have positive values.

STEP 1006: Label each such local maximum as an ion.

STEP 1008: Extract ion parameters.

STEP 1010: Tabulate ion parameters.

STEP 1012: Post-process ion parameters to obtain molecular

properties.

[0096] In Step 1008, the parameters of each ion are obtained by examining the maximal element. An ion's retention time is the time of the scan containing the maximal element. The ion's m/z is the m/z for the channel containing the maximal element. The ion's intensity is the intensity of the maximal element itself, or alternatively, the intensity can be the sum of intensities of elements surrounding the maximal element. Interpolation techniques, described below, can be used to better estimate these parameters. Secondary observable parameters, including for example, the widths of the peak in the chromatographic and spectral directions, can also be determined.

Steps 2 and 3: Specification and Application of Filter

Need for filters

[0097] Rarely, if ever, is co-elution, interference, or noise absent in LC/MS experiments. The presence of co-elution, interference, or noise can severely

reduce the ability to accurately and reliably detect ions. Consequently, the simple detection and quantification procedure illustrated by flow chart 1000 may not be adequate in all circumstances.

Coelution

[0098] Figure 11 is an exemplary contour plot showing the effects of co-elution and interference due to finite peak widths. In the example illustrated in Figure 11, another ion, ion 4, is assumed to have m/z and retention time values somewhat larger than that of ion 1 as well as have an apex in both the spectral and chromatographic directions lying within the FWHM of the apex of ion 1. As a result, ion 4 is co-eluted with ion 1 in the chromatographic direction and interferes with ion 1 in the spectral direction.

[0099] Figure 12 illustrates the spectral effects due to co-elution of ion 4 at the times indicated by lines A, B, and C of Figure 11. In each spectrum shown in Figure 12, ion 4 appears as a shoulder to ion 1. This is also apparent from the contour plot shown in Figure 11 because there is no distinct apex associated with ion 4.

[0100] Thus one problem with detection in LC/MS systems is that pairs of ions may co-elute in time and interfere spectrally such that the pair of ions produces only a single local maximum, not two. Co-elution or interference can cause true ions, having significant intensity in the data matrix, to be missed, *i.e.*, not detected. Such missed detection of a true peak as an ion is referred to as a false negative.

Noise

[0101] Noise encountered in LC/MS systems typically falls into two categories: detection noise and chemical noise. Detector and chemical noise combine to establish a baseline noise background against which the detection and quantitation of ions is made.

[0102] Detection noise, also known as shot or thermal noise, is inherent in all detection processes. For example, counting detectors, such as MCPs, add shot noise, and amplifiers, such as electrometers, add thermal or Johnson noise. The statistics of shot noise are generally described by Poisson statistics. The statistics of Johnson noise are generally described by

Gaussian statistics. Such detection noise is inherent in the system and cannot be eliminated.

[0103] The second kind of noise encountered in LC/MS systems is chemical noise. Chemical noise arises from several sources. For example, small molecules that are inadvertently caught up in the process of separation and ionization can give rise to chemical noise. Such molecules can be a constant presence, each producing an essentially constant background intensity at a given mass-to-charge ratio, or each such molecule can be separated thereby producing a chromatographic profile at a characteristic retention time. Another source of chemical noise is found in complex samples, which can contain both molecules whose concentrations vary over a wide dynamic range and interfering elements whose effects are more significant at lower concentrations.

[0104] Figure 13 is an exemplary contour plot illustrating the effects of noise. In Figure 13, numerically generated noise is added to an ion peak contour plot to simulate the effects of chemical and detector noise. Figure 14A illustrates mass spectra (Spectra A, B and C) corresponding to lines A, B and C respectively in Figure 13, Figure 14B illustrates chromatograms for ions 1, 2, 3 corresponding to lines labeled ion 1, ion 2 and ion 3 respectively in Figure 13. As can be seen in Figure 13, one detrimental effect of the additive noise is that it causes apices to appear throughout the plot, including within the FWHM of the nominal apex locations associated with ions 1 and 2. These noise-induced apices can be erroneously identified as peaks corresponding to ions, thereby resulting in false positive ion detections.

[0105] Thus, local maxima may be due to the noise rather than ions. As a result, false peaks, *i.e.*, peaks not associated with an ion, may be counted as an ion. Moreover, noise might produce more than one multiple local maximum for an ion. These multiple maxima could result in detection of peaks that do not represent true ions. Thus, peaks from a single ion could be multiply counted as separate ions when in fact the multiple peaks are due only to a single ion. Such detection of false peaks as ions is referred to as false positives.

[0106] In addition to disregarding noise effects, the simple ion detection algorithm described in Figure 10 is generally not statistically optimal. This is because the variance in the estimates of retention time, m/z and intensity are determined by the noise properties of a single maximal element. The simplified algorithm does not make use of the other elements in the island of intensities surrounding the maximal element. As described in more detail below, such neighboring elements can be used to reduce variance in the estimate.

Role of Convolution

[0107] According to embodiments of the present invention, the LC/MS data matrix is a two-dimensional array. Such a data matrix can be processed by convolving it with a two-dimensional array of filter coefficients.

[0108] The convolution operation employed in embodiments of the present invention provides a more general and powerful approach to peak detection than the simple signal-averaging schemes employed in conventional systems. The convolution operation employed in embodiments of the present invention addresses the limitations of the method described in Figure 10.

[0109] The filter coefficients can be chosen to provide estimates of ion parameters that have better signal-to-noise ratios than those obtained from analyzing single channels or scans.

[0110] The convolution filter coefficients can be chosen to produce estimates of ion parameters that have the greatest precision or least statistical variance for a particular data set. These benefits of embodiments of the present invention provide more reproducible results for ions at low concentration than do conventional systems.

[0111] Another advantage of embodiments of the present invention is that filter coefficients can be chosen to resolve ions that are co-eluted and interfering. For example, the apices of ions appearing as shoulders to other ions in a mass spectrum can be detected using appropriately specified filter co-efficients in embodiments of the present invention. Such detection overcomes limitations associated with conventional techniques in analyzing

complex chromatograms, where co-elution and ion-interference are a common problem.

[0112] Another advantage of embodiments of the present invention is that filter coefficients can be chosen to subtract baseline signals, producing more accurate estimates of ion intensity.

[0113] Another advantage of embodiments of the present invention is that filter coefficients can be chosen to minimize the computation burden of convolution, resulting in high-speed operation of peak detection and the estimation of ion parameters.

[0114] In general, numerous filter shapes can be used in the convolution, including, for example, Savitzky-Golay (SG) smoothing and differentiating filters. The filter shapes can be chosen to perform a number functions including smoothing, peak identification, noise reduction and baseline reduction. Filter shapes used in preferred embodiments of the present invention are described below

Implementation of convolution in this invention

[0115] The convolution operation according to embodiments of the present invention is linear, non-iterative and not dependent on the values of the data in the data matrix. In an embodiment of the present invention, the convolution operation is implemented by means of a general purpose programming language using a general purpose computer such as computer 118. In an alternate embodiment of the present invention, the convolution operation is implemented in a special purpose processor known as digital-signal-processor (DSP). Typically, DSP-based filtering provides enhanced processing speed over general purpose computer-based filtering.

[0116] In general, convolution combines two inputs to produce one output. Embodiments of the present invention employ a two-dimensional convolution. One input to the two-dimensional convolution operation is the data matrix of intensities created from the spectral output of an LC/MS experiment. The second input to the two-dimensional convolution operation is a matrix of filter coefficients. The convolution operation outputs an output convolved matrix. Generally, the output convolved matrix has the same number of rows and column elements as the input LC/MS matrix.

[0117] For simplicity in the present description, assume that the LC/MS data matrix is rectangular and that the size of the matrix of filter coefficients is comparable to the size of a peak. In this case the size of the filter coefficient matrix is smaller than the size of the input data matrix or output convolved matrix.

[0118] An element of the output matrix is obtained from the input LC/MS data matrix as follows: the filter matrix is centered on an element in the input data matrix, and then the input data matrix elements are multiplied by the corresponding filter matrix elements and the products are summed, producing an element of the output convolved data matrix. By combining neighboring elements, convolution filters reduce variance in the estimates of an ion's retention time, mass-to-charge ratio, and intensity.

[0119] The edge-values of the output convolved matrix are those elements that are within half the filter width from the edge of the output convolved matrix. Generally these elements can be set to an invalid value in embodiments of the present invention to indicate invalid filtering values. Generally, ignoring these edge values is not a significant limitation for embodiments of the present invention and these invalid values can be ignored in subsequent processing.

One-dimensional Convolution

[0120] Convolution for a one-dimensional case is clearly described in detail. This description is followed by generalizing convolution to the two-dimensional case. It is useful to first describe the one-dimensional case because the two-dimensional convolution operation that is used in the preferred embodiment of the present invention is implemented by applying a series of one-dimensional convolutions to the data matrix.

[0121] In one dimension, the convolution operation is defined as follows. Given a one-dimensional, N -element, input array of intensities d_i and a one-dimensional, M -element, array of convolution filter coefficients f_j , the convolution operation is defined as:

$$c_i = \sum_{j=-h}^h f_j d_{i-j} \quad (1)$$

where c_i is the output convolved array, and $i = 1, \dots, N$. For convenience, M is chosen to be an odd number. The index j varies from $j = -h, \dots, 0, \dots, h$, where h is defined as $h \equiv (M-1)/2$.

[0122] Thus, the value of c_i corresponds to a weighted sum of the h elements surrounding d_i . Spectra and chromatograms are examples of one-dimensional input arrays that contain peaks. The width of the convolution filter f_j is set to be approximately the width of a peak. Thus, M is on the order of the number of array elements that span the width of a peak. Peaks have a width which typically is much smaller than the length N of the input array, so that in general $M \ll N$.

[0123] Although the index i for d_i ranges from 1 to N , in some embodiments of the present invention, c_i is defined only for $i > h$ or $i \leq (N-h)$ to account for edge effects. The value for c_i near the array boundaries, *i.e.* when $i \leq h$ or $i > (N-h)$, is not defined for the summation. Such edge effects can be handled by limiting the values for c_i to be $i > h$ or $i \leq (N-h)$, where the summation is defined. In this case, the summation applies only to those peaks far enough away from the array edges so that the filter f_j can be applied to all points within the neighborhood of the peak. That is, filtering is not performed at the edges of the data array d_i . Generally, ignoring edge effects is no a significant limitation for embodiments of the present invention.

[0124] If filtered values are needed near the edge for $1 < i < h$ or $N \geq i > (N-h)$, either the data array and/or the filter coefficients can be modified for these edge elements. The data matrix can be modified by append h elements to each end the array, and apply the M coefficient filter to an array that contains $N + 2h$ elements.

[0125] Alternatively, edge effects can be considered by appropriately modifying the limits of the filtering function to account for there being less than M points for filtering near the edges.

Two-dimensional Convolution

[0126] The one-dimensional convolution operation described above can be generalized to the case of two-dimensional data for use in embodiments of the present invention. In the two-dimensional case, one input to the convolution operation is a data matrix $d_{i,j}$ subscripted by two indices, (i, j) , wherein $i = 1, \dots, M$ and $j = 1, \dots, N$. The data values of the input data matrix can vary from experiment to experiment. The other input to the convolution is a set of fixed filter coefficients, $f_{p,q}$, that is also subscripted by two indices. The filter coefficients matrix, $f_{p,q}$, is a matrix that has $P \times Q$ coefficients. Variables h and l are defined as $h \equiv (P-1)/2$ and $l \equiv (Q-1)/2$. Thus, $p = -h, \dots, h$, and $q = -l, \dots, l$.

[0127] Convolution of $d_{i,j}$ with $f_{p,q}$ yields the output convolved matrix $c_{i,j}$:

$$c_{i,j} = \sum_{p=-h}^h \sum_{q=-l}^l f_{p,q} d_{i-p, j-q}. \quad (2)$$

[0128] Generally, the size of the filter is much less than the size of the data matrix, so that $P \ll M$ and $Q \ll N$. The above equation indicates that $c_{i,j}$ is computed by centering $f_{p,q}$ on the (i,j) th element of $d_{i,j}$ and then using the filter coefficients $f_{p,q}$ to obtain the weighted sum of the surrounding intensities. Thus, each element of the output matrix $c_{i,j}$ corresponds to a weighted sum of the elements of $d_{i,j}$, wherein each element $d_{i,j}$ is obtained from a region centered on the i,j th element.

[0129] Although the index i and j for $d_{i,j}$ ranges from $i=1$ to N , and j from 1 to M , in some embodiments of the present invention, $c_{i,j}$ is defined only for $i \geq h$ or $i \leq (N-h)$ and $j \geq l$ or $j \leq (M-l)$ to account for edge effects. The value for c_i near the array boundaries, i.e. when $i < h$ or $i > (N-h)$ and/or $j < l$ or $j > (M-l)$ is not defined for the summation. Such edge effects can be handled by limiting the values for $c_{i,j}$ to be those where the summation is defined. In this case, the summation applies only to those

peaks far enough away from the array edges so that the filter $f_{p,q}$ can be applied to all points within the neighborhood of the peak. That is, filtering is not performed at the edges of the data array $d_{i,j}$. Generally, ignoring edge effects is no a significant limitation for embodiments of the present invention.

[0130] If filtered values are needed near the edge for $1 \leq i < h$ and $N \geq i > (N - h)$, either the data matrix and/or the filter coefficients matrix can be modified for these edge elements. One approach is to append h elements to the end of each row, and l elements to the end of each column. The two-dimensional convolution filter is then applied to a data matrix that contains $(N + 2h) \times (M + 2l)$ elements.

[0131] Alternatively, edge effects can be considered by appropriately modifying the limits of the filtering function to account for there being less than P points for filtering near the row edges and Q points for filtering near the column edges.

[0132] The computational burden for implementation of equation (2) can be determined as follows. If $f_{p,q}$ contains $P \times Q$ coefficients then the number of multiplications needed to compute a value for $c_{i,j}$ is $P \times Q$. For example, where $P = 20$ and $Q = 20$, it follows that 400 multiplications are needed to determine each output point $c_{i,j}$ in the output convolved matrix. This is a high computation burden that can be eased by other approaches to two-dimensional convolution.

Two-dimensional Convolution with Rank-1 Filters

[0133] The two-dimensional convolution filter described in equation (2) applies a filter matrix that contains $P \times Q$ independently specified coefficients. There are other ways for specifying the filter coefficients. Although the resulting convolution coefficients are not as freely specified, the computation burden is eased.

[0134] One such alternate way of specifying the filter coefficients is as a rank-1 filter. To describe a rank-1 convolution filter, consider that a two-dimensional convolution of the LC/MS data matrix can be accomplished by

the successive application of two one-dimensional convolutions. See for example, in JOHN H. KARL, INTRODUCTION TO DIGITAL SIGNAL PROCESSING, PG. 320 (ACADEMIC PRESS 1989) ("KARL"), which is hereby incorporated by reference herein. For example, a one-dimensional filter, g_q , is applied to each row of the LC/MS data matrix, producing an intermediate convolved matrix. To this intermediate convolved matrix, a second one-dimensional filter, f_p , is applied to each column. Each one-dimensional filter can be specified with a different set of filter coefficients. Equation (3) illustrates how the filters comprising a rank-1 convolution filter are applied in succession, wherein the intermediate matrix is enclosed in the parentheses.

$$c_{i,j} = \sum_{p=-h}^h f_p \left(\sum_{q=-l}^l g_q d_{i-p,j-q} \right) \quad (3)$$

$$= \sum_{p=-h}^h \sum_{q=-l}^l f_p g_q d_{i-p,j-q}. \quad (4)$$

[0135] The computational burden for implementation of equation (3) can be determined as follows. If f_p contains P coefficients and g_q contains Q coefficients, then the number of multiplications needed to compute a value for $c_{i,j}$ is $P+Q$. For example, where $P=20$ and $Q=20$, only 40 multiplications are needed to determine each output point $c_{i,j}$ in the output convolved matrix. As can be seen, this is more computationally efficient than the general case of two-dimensional convolution described in Eq. (2) where $20 \times 20 = 400$ are required to determine each $c_{i,j}$.

[0136] Equation (4) is a rearrangement of equation (3) that illustrates that the successive operations are equivalent to a convolution of the data matrix with a single coefficient matrix whose elements are pair-wise products of the one dimensional filters. An examination of equation (4) shows that in using the rank-1 formulation, the effective two-dimensional convolution matrix is a rank-1 matrix formed by the outer product of two one-dimensional vectors. Thus, equation (4) can be rewritten as:

$$c_{i,j} = \sum_{p=-h}^h \sum_{q=-l}^l F_{pq} d_{i-p,j-q} \quad (5)$$

$$F_{pq} \equiv f_p g_q. \quad (6)$$

The two-dimensional coefficient matrix F_{pq} emerges from the convolution operation. F_{pq} has the form of a rank-1 matrix, where a rank-1 matrix is defined as the outer product of a column vector (here, f_p) and a row vector (here, g_q). See for example, in GILBERT STRANG, INTRODUCTION TO APPLIED MATHEMATICS, 68FF (WELLESLEY-CAMBRIDGE PRESS 1986) ("STRANG"), which is hereby incorporated by reference herein.

[0137] In embodiments of the present invention using a rank-1 filter implementation, the rank-1 filter is characterized by two orthogonal cross sections, one for each filter. The filter for each orthogonal cross-section is specified by a one-dimensional filter array.

Two-dimensional Convolution with Rank-2 Filters

[0138] A two-dimensional convolution operation can be carried out with a rank-2 filter. Two-dimensional convolution with a rank-2 filter is carried out by computing two rank-1 filters and summing their result. Thus, four filters: f_p^1, g_q^1, f_p^2 , and g_q^2 are required to implement a rank-2 filter for the two-dimensional convolution performed in embodiments of the present invention.

[0139] Two of the filters f_p^1 and g_q^1 are associated with the first rank-1 filter and two of the filters f_p^2 and g_q^2 are associated with the second rank-1 filter. These four filters f_p^1, f_p^2 and g_q^1, g_q^2 are implemented as follows:

$$c_{i,j} = \sum_{p=-h}^h f_p^1 \left(\sum_{q=-l}^l g_q^2 d_{i-p,j-q} \right) + \sum_{p=-h}^h f_p^2 \left(\sum_{q=-l}^l g_q^1 d_{i-p,j-q} \right) \quad (7)$$

$$= \sum_{p=-h}^h \sum_{q=-l}^l (f_p^1 g_q^1 + f_p^2 g_q^2) d_{i-p,j-q} \quad (8)$$

[0140] Filters f_p^1 and f_p^2 are applied in the spectral direction (along the columns) and filters g_q^1 and g_q^2 are applied in the chromatographic direction

(along the rows). Equation (7) illustrates how each filter pair can be applied in succession, where the intermediate matrix is enclosed in the braces, and how the results from the two rank-1 filters are summed. Equation (7) shows the preferred manner of implementing the rank-2 filter according to embodiments of the present invention.

[0141] Equation (8) is a rearrangement of equation (7) to show that the successive operations in the rank-2 filter configuration are equivalent to a convolution of the data matrix with a single coefficient matrix whose elements are the sum of pair-wise products of the two one-dimensional filter pairs.

[0142] To analyze the computational requirements of a rank-2 filter, consider that if f_p^1 and f_p^2 both contain P coefficients and g_q^1 and g_q^2 both contain Q coefficients, then the number of multiplications needed to compute a value for an element of the output convolution matrix $c_{i,j}$ is $2(P+Q)$. Thus, in the case where $P=20$ and $Q=20$, only 80 multiplications are needed to compute each element of the output convolution matrix, whereas in the general case as shown in equation (2), $20 \times 20 = 400$ are required to compute each $c_{i,j}$.

[0143] Thus, an embodiment of the present invention employing a rank-2 filter, the effective two-dimensional convolution matrix is formed from the sum of the outer product of two pairs of one-dimensional vectors. Equation (8) can be rewritten as

$$c_{i,j} = \sum_{p=-h}^h \sum_{q=-l}^l F_{pq} d_{i-p,j-q} \quad (9)$$

$$F_{pq} \equiv f_p^1 g_q^1 + f_p^2 g_q^2 \quad (10)$$

[0144] Two-dimensional coefficient matrix F_{pq} emerges from the convolution operation. The two-dimensional coefficient matrix F_{pq} has the form of a rank-2 matrix, where a rank-2 matrix is defined as the sum of two linearly independent rank-1 matrices as described in STRANG. Here $f_p^1 g_q^1$ and $f_p^2 g_q^2$ are each rank-1 matrices.

Filter Specifications

- [0145] Equations (2), (3), and (7) are all embodiments of two-dimensional convolution filters of the present invention. Equation (2) specifies the filter coefficients as a matrix $f_{p,q}$, equation (3) specifies the filter coefficients as a set of two one-dimensional filters, f_p and g_q , and equation (7) specifies the filters as a set of four one-dimensional filters, f_p^1 , g_q^1 and f_p^2 , g_q^2 .
- [0146] Equations (2), (3), and (7) do not specify the preferred values of these coefficients. The values of the filter coefficients for the present invention are chosen to address the limitations of the method of Figure 10. The filter coefficients are chosen to accomplish several goals which include the reduction of the effects of detector and chemical noise, the partial resolution of coeluted and interfered peaks, the subtraction of baseline noise, and achievement of computational efficiency and high-speed operation.
- [0147] The Matched Filter Theorem (MFT) is a prescriptive method, known in the prior art, to obtain filter coefficients than can be implemented using Equation (2). See for example, KARL at 217; BRIAN D.O. ANDERSON & JOHN B. MOORE, OPTIMAL FILTERING 223ff (PRENTICE-HALL INC. 1979)("ANDERSON") at 223ff which is hereby incorporated by referral herein. Filters obtained from the MFT are designed to detect the presence of signals and to reduce the effects of detector noise. Such filters can then be used to detect ions in the LC/MS data matrix and can be used to determine the retention time, mass-to-charge ratio, and intensity of ions. A filter obtained from the MFT is an improvement over the method of Figure 10. In particular such filters reduce variance and improve precision by combining data from elements within a peak that neighbor the peak apex. However, such filters are not designed to subtract baseline noise or to resolve coeluted and interfered peaks. Filters obtained from the MFT and are not designed to achieve high speed operation.
- [0148] The MFT and a set of filter coefficients that can be obtained from it represent an improvement over the method of Figure 10 are described, then modified filters that subtract baselines, reduce the effects of coelution and interference, while still reducing the effect of detector and chemical noise

are described. Such filters employ a combination of smoothing and second-derivative filters and are implemented using Equations (3) and (7). The preferred embodiment uses equation (7) with a combination of smoothing and second-derivative filters that together reduce noise, resolve interfering peaks, subtract baselines, and reduce the computational burden to allow for high-speed operation.

Matched Filter Theorem for One-dimensional convolution

[0149] The MFT is first described for one-dimensional convolution. It is then generalized to two-dimensional convolution.

[0150] Coefficients for f_j are chosen to perform a detection function. For example, the matched filter theorem (MFT) provides a set of filter coefficients known as a matched filter that can be used to perform the detection function.

[0151] The MFT assumes that the data array d_i can be modeled as a sum of a signal $r_o s_i$ plus additive noise, n_i :

$$d_i = r_o s_{i-i_o} + n_i.$$

The shape of the signal is fixed and described by a set of coefficients, s_i . The scale factor r_o determines the signal amplitude. The MFT also assumes that the signal is bounded. That is, the signal is zero (or small enough to be ignored) outside some region. The signal is assumed to extend over M elements. For convenience, M is typically chosen to be odd and the center of the signal is located at s_o . If h is defined as $h \equiv (M-1)/2$, then $s_i = 0$ for $i < -h$ and for $i > h$. In the above expression, the center of the signal appears at $i = i_o$.

[0152] For purposes of simplifying the present description the noise elements n_i are assumed to be uncorrelated Gaussian deviates, with zero mean and a standard deviation of σ_o . More general formulations for the MFT accommodate correlated or colored noise. See example, ANDERSON at 288-304.

[0153] Under these assumptions, the signal-to-noise ratio (SNR) of each element is $r_o s_i / \sigma_o$. The SNR of a weighted sum of the data that contains the signal s_i can be determined by considering an M-element set of weights w_i , centered to coincide with the signal where $h \equiv (M-1)/2$, and $i = -h, \dots, 0, \dots, h$. Assuming the weights are centered to coincide with the signal, the weighted sum S is defined as:

$$S = \sum_{i=-h}^h w_i d_{i-i_o} = r_o \sum_{i=-h}^h w_i s_i + \sum_{i=-h}^h w_i n_{i-i_o}.$$

[0154] The mean value of the noise term in an ensemble average is zero. Consequently, the average value of S over an ensemble of arrays, where the signal in each array is the same, but the noise is different is:

$$\langle S \rangle = r_o \sum_{i=-h}^h w_i s_i.$$

[0155] To determine the noise contribution, the weights are applied to a region containing only noise. The ensemble mean of the sum is zero. The standard deviation of the weighted sum about the ensemble mean is:

$$\sigma \equiv \sqrt{\langle (S - \langle S \rangle)^2 \rangle} = \sigma_o \sqrt{\sum_{i=-h}^h w_i^2}.$$

[0156] Finally, the SNR is determined as:

$$\frac{\langle S \rangle}{\sigma} = \frac{r_o \left(\sum_{i=-h}^h w_i s_i \right)}{\sigma_o \sqrt{\sum_{i=-h}^h w_i^2}}.$$

This result is for a general set of weighting coefficients w_i .

[0157] The MFT specifies values for w_i that maximize the SNR. If the weighting factors w_i are regarded as elements of an M dimensional vector w of unit length, *i.e.*, the weighting factors are normalized so that $\sqrt{\sum_{i=-h}^h w_i^2} = 1$,

then the SNR is maximized when the vector w points in the same direction as the vector s . The vectors point in the same direction when respective elements are proportional to each other *i.e.*, when $w_i \propto s_i$. Consequently, the MFT implies that the weighted sum has the highest signal-to-noise when the weighting function is the shape of the signal itself.

[0158] If w_i is chosen such that $w_i = s_i$, then for noise with unit standard deviation, the SNR reduces to:

$$\frac{\langle S \rangle}{\sigma} = \frac{r_o}{\sigma_o} \frac{\left(\sum_{i=-h}^h s_i^2 \right)}{\sqrt{\sum_{i=-h}^h s_i^2}} = \frac{r_o}{\sigma_o} \sqrt{\sum_{i=-h}^h s_i^2}.$$

This formulation of SNR corresponds to the signal properties of the weighted sum when the filter coefficients are centered on the signal and the noise properties when the filter is in a noise-only region.

Matched Filter Theorem for Two-dimensional convolution

[0159] The MFT discussed above for the one-dimensional case can also be generalized to the two-dimensional case for a bounded, two-dimensional signal embedded in a two-dimensional array of data. As before, the data is assumed to be modeled as a sum of signal plus noise:

$$d_{i,j} = r_o s_{i-i_o, j-j_o} + n_{i,j},$$

wherein the signal $S_{i,j}$ is limited in extent and whose center is located at (i_o, j_o) with amplitude r_o . Each noise element $n_{i,j}$ is an independent Gaussian deviate of zero mean and standard deviation σ_o .

[0160] To determine the SNR of a weighted sum of the data that contains the signal $S_{i,j}$ consider a $P \times Q$ -element set of weights $w_{i,j}$, wherein $h = (P-1)/2$ and $l = (Q-1)/2$, such that $i = -h, \dots, h$, and $j = -l, \dots, l$. The weights are centered to coincide with the signal. The weighted sum S is:

$$S = \sum_{i=-h}^h \sum_{j=-l}^l w_{i,j} d_{i-i_o, j-j_o} = r_o \sum_{i=-h}^h \sum_{j=-l}^l w_{i,j} s_{i,j} + \sum_{i=-h}^h \sum_{j=-l}^l w_{i,j} n_{i-i_o, j-j_o}.$$

[0161] The average value of S over the ensemble is:

$$\langle S \rangle = r_o \sum_{i=-h}^h \sum_{j=-l}^l w_{i,j} s_{i,j}.$$

The standard deviation of the noise is:

$$\sigma = \sigma_o \sqrt{\sum_{i=-h}^h \sum_{j=-l}^l w_{i,j}^2}.$$

and the signal-to-noise ratio is:

$$\frac{\langle S \rangle}{\sigma} = \frac{r_o \sum_{i=-h}^h \sum_{j=-l}^l w_{i,j} s_{i,j}}{\sigma_o \sqrt{\sum_{i=-h}^h \sum_{j=-l}^l w_{i,j}^2}}$$

[0162] As in the one-dimensional case described above, the SNR is maximized when the shape of the weighting function is proportional to the signal, that is when $w_{i,j} \propto s_{i,j}$. The signal properties of the weighted sum correspond to where the filter coefficients are centered on the signal, and the noise properties of the weighted sum correspond to where the filter is in the noise-only region.

[0163] The Matched Filter achieves maximum signal-to-noise by optimally combining neighboring elements. Convolution filters that employ matched filter coefficients produce minimum variance in the estimates of an ion's retention time, mass-to-charge ratio, and intensity.

Matched filters guaranteed to produce unique maximum

[0164] In general, signal detection using convolution proceeds by moving the filter coefficients along the data array and obtaining a weighted sum at each point. For example, where the filter coefficients satisfy MFT, i.e., $w_i = s_i$ (the filter is matched to the signal) then in the noise-only region of the data, the amplitude of the output is dictated by the noise. As the filter overlaps the signal, the amplitude increases, and must reach a unique maximum when the filter is aligned in time with the signal.

One-dimensional Gaussian Matched Filter

[0165] As an example of the foregoing technique for one-dimensional convolution, consider the case where the signal is a single peak resulting from a single ion. The peak (spectral or chromatographic) can be modeled as a Gaussian profile whose width is given by the standard deviation σ_p , where the width is measured in units of sample elements. The signal is then:

$$r_i = r_o \exp\left(-\frac{1}{2} \frac{(i-i_o)^2}{\sigma_p^2}\right).$$

[0166] Assume the filter boundary is set to $\pm 4\sigma_p$. According to the Matched Filter Theorem, the filter is the signal shape itself, *i.e.*, a Gaussian, centered on zero and bounded by $\pm 4\sigma_p$. The coefficients of such a matched filter are given by:

$$f_i = \exp\left(-\frac{1}{2} \frac{i^2}{\sigma_p^2}\right), \text{ for } i > -4\sigma_p \text{ and } i < 4\sigma_p.$$

[0167] Assume further that the system samples four points per standard deviation. As a result, $\sigma_p = 4$, so $i = -16, \dots, 16$, and the filters are 33 points wide for the present example. For the Gaussian matched filter (GMF) in one-dimension, the maximum signal of the convolved output array is $7.09 r_o$, and the noise amplitude is $2.66 \sigma_o$. The SNR associated with using the matched filter is $2.66 (r_o / \sigma_o)$.

Gaussian Matched Filter contrasted with Boxcar Filter in one dimension

[0168] We contrast the GMF with a simple boxcar filter in one-dimension. Again, the signal is assumed to be a peak that is modeled by Gaussian shape described above. Assume the filter boundary for the boxcar is also set to $\pm 4\sigma_p$. The coefficients of the boxcar filter are given by:

$$f_i = \frac{1}{M} = \frac{1}{8\sigma_p + 1}.$$

The output of the boxcar filter is the average value of the input signal over M points ($M = 8\sigma_p + 1$).

[0169] Again, assume further that the system samples four points per standard deviation, so the boxcar filter is 33 points wide. For a Gaussian peak of unit height, the average signal over the peak using the boxcar filter is $0.304 r_o$, and the standard deviation of the noise is $\sigma_o / \sqrt{33} = 0.174 \sigma_o$. The SNR using the boxcar filter is $1.75 (r_o / \sigma_o)$.

[0170] Thus, the SNR of the Gaussian matched filter relative to the boxcar is $2.66/1.75=1.52$, or more than 50% higher than that provided by the boxcar filter.

[0171] Both the matched filter and the boxcar filter are linear. The convolution of either of these filters with the Gaussian peak shape produces an output that has a unique maximum value. Thus, either of these filters can be used in the convolution of embodiments of the present invention. However, in the case of Gaussian noise, because of its higher SNR at the local maximum, the matched filter is preferred.

Gaussian noise and Poisson noise

[0172] The Gaussian Matched Filter is an optimum filter when the noise has Gaussian statistics. For counting detectors the boxcar filter will be optimal because it is simply a sum of all counts associated with a peak. In order to sum all the counts associated with a peak the width of the boxcar filter should be related to the width of the peak. Typically the width of the boxcar filter will be between 2 and 3 times the FWHM of the peak.

Two-dimensional Gaussian Matched Filter

[0173] As an example of the Matched Filter technique for two-dimensional convolution, consider the case where the signal is a single peak resulting from a single ion. The peak can be modeled as a Gaussian profile in both the spectral and chromatographic directions. The spectral width is given by the standard deviation σ_p , where the width is measured in units of sample

elements, and the chromatographic width is given by the standard deviation σ_q , where the width is measured in units of sample elements. The signal, centered on data matrix element i_o, j_o is then:

$$r_{i,j} = r_o \exp\left(-\frac{1}{2} \frac{(i-i_o)^2}{\sigma_p^2}\right) \exp\left(-\frac{1}{2} \frac{(j-j_o)^2}{\sigma_q^2}\right).$$

[0174] Assume the filter boundary is set to $\pm 4\sigma_p$ and $\pm 4\sigma_q$. According to the Matched Filter Theorem, the filter is the signal shape itself, *i.e.*, a Gaussian, centered on zero and bounded by $\pm 4\sigma_p$ and $\pm 4\sigma_q$. The coefficients of such a matched filter are given by:

$$f_{p,q} = \exp\left(-\frac{1}{2} \frac{p^2}{\sigma_p^2}\right) \exp\left(-\frac{1}{2} \frac{q^2}{\sigma_q^2}\right),$$

for $p > -4\sigma_p$ and $p < 4\sigma_p$ and $q > -4\sigma_q$ and $q < 4\sigma_q$

[0175] Assume further that the system samples four points per standard deviation for both the spectral and chromatographic directions. As a result, $\sigma_p = 4$ and $\sigma_q = 4$, so that $p = -16, \dots, 16$ and $q = -16, \dots, 16$, and the filters are 33×33 points for the present example. For the Gaussian matched filter (GMF) in two-dimensions, the maximum signal in the convolved output matrix is $50.3 r_o$, and the noise amplitude is $7.09 \sigma_o$. The SNR associated with using the matched filter is $7.09 (r_o / \sigma_o)$.

[0176] A two-dimensional convolution filter performs a filter operation on the LC/MS data matrix in both the chromatographic and in the mass spectrometric directions. As a result of the convolution operation, the output convolution matrix will contain peaks whose shapes are, in general, widened or other wise distorted relative to the input LC/MS data matrix. In particular the Matched Gaussian Filter will always produce peaks in the output convolution matrix that are widened by a factor of $\sqrt{2}$ in both the chromatographic and spectral directions relative to the input peaks.

[0177] At first glance, it may seem that the widening produced by the GMF may be detrimental to the accurate estimate of critical parameters of retention time, mass-to-charge ratio, or intensity. But the Matched Filter Theorem shows that two-dimensional convolution produces apex values whose retention time, mass-to-charge ratio and intensity result form the effective combination of all spectral and chromatographic elements associated with the peak such that the resulting apex-associated values produce statistically optimum estimates of retention time, m/z, and intensity for that peak.

Gaussian Matched Filter contrasted with Boxcar Filter in two-dimensions

[0178] We contrast the GMF with a simple boxcar filter in two-dimension. Again, the signal is assumed to be a peak that is modeled by Gaussian shape described above. Assume the filter boundary for the boxcar is also set to $\pm 4\sigma_p$. The coefficients of the boxcar filter are given by:

$$f_{i,j} = \frac{1}{M \times N} = \frac{1}{8\sigma_p + 1} \times \frac{1}{8\sigma_q + 1}.$$

The output of the boxcar filter is the average value of the input signal over $M \times N$ points.

[0179] Again, assume further that the system samples four points per standard deviation, so the boxcar filter is 33 x 33 points wide. For a Gaussian peak of unit height, the average signal over the peak using the boxcar filter is 0.092 r_o , and the standard deviation of the noise is 0.303 σ_o . The SNR using the boxcar filter is 3.04 (r_o / σ_o).

[0180] Thus, the SNR of the Gaussian matched filter relative to the boxcar is 7/3 = 2.3, or more than twice that provided by the boxcar filter.

[0181] Both the matched filter and the boxcar filter are linear. The convolution of either of these filters with the Gaussian peak shape produces an output that has a unique maximum value. Thus, either of these filters can be used in the convolution of embodiments of the present invention.

However, in the case of Gaussian noise, because of its higher SNR at the local maximum, the matched filter is preferred.

Gaussian noise and Poisson noise

[0182] The Gaussian Matched Filter in two-dimensions is an optimum filter when the noise has Gaussian statistics. For counting detectors the boxcar filter will be optimal because it is simply a sum of all counts associated with a peak. In order to sum all the counts associated with a peak the widths of the boxcar filter should be related to the width of the peak in the spectral and chromatographic directions. Typically the widths of the boxcar filter will be between 2 and 3 times the respective FWHMs of the peak in the spectral and chromatographic directions.

Gaussian Matched Filter for the detection of ions in LC/MS data matrix

[0183] For the Gaussian Matched Filter, the specification (Step 2) of the two-dimensional convolution filter is the coefficients are Gaussian filter coefficients $f_{p,q}$ as described above, and the application (Step 3) of the filter is then according to Eq. (2) using these filter coefficients. This embodiment of Step 2 and Step 3 then provides a method to detect ions, and to determine their retention time, mass-to-charge ratio, and intensity. The results from such a method reduce the effects of detector noise and are an improvement over the method of Figure 10.

Filters coefficients that are not matched filters.

[0184] Linear weighting coefficients other than those that follow the signal shape can also be used. While such coefficients may not produce the highest possible SNR, they may have other counter-balancing advantages. The advantages include the ability to partially resolve coeluted and interfered peaks, the subtraction of baseline noise, and computational efficiency leading to high-speed operation. We analyze the limitations of the Gaussian Matched Filter and describe linear filter coefficients that address these limitations.

Issues with Gaussian Matched filters

[0185] For a Gaussian peak, the Matched Filter Theorem (MFT) specifies the Gaussian Matched Filter (GMF) as the filter whose response has the highest signal-to-noise ratio as compared to any other convolution filter.

However, the Gaussian Matched Filter (GMF) may not be optimal in all cases.

[0186] One disadvantage of the GMF is that it produces a widened or broadened output peak for each ion. To help explain peak broadening, it is well known that if a signal having positive values and a standard width, σ_s , is convolved with a filter having positive values and a standard width, σ_f , the standard width of the convolved output is increased. The signal and filter width combine in quadrature to produce an output width of $\sigma_o = \sqrt{\sigma_s^2 + \sigma_f^2}$. In the case of the GMF, where the widths of the signal and filter are equal, the output peak is wider than the input peak by a factor of approximately $\sqrt{2} \approx 1.4$, i.e., 40%.

[0187] Peak broadening can cause the apex of a small peak to be masked by a large peak. Such masking could occur, for example, when the small peak is nearly co-eluted in time and nearly coincident in mass-to-charge with the larger peak. One way to compensate for such co-elution is to reduce the width of the convolution filter. For example, halving the width of the Gaussian convolution filter produces an output peak that is only 12% more broad than the input peak. However, because the peak widths are not matched, the SNR is reduced relative to that achieved using a GMF. The disadvantage of reduced SNR is offset by the advantage of increased ability to detect nearly coincident peak pairs.

[0188] Another disadvantage of the GMF is that it has only positive coefficients. Consequently, the GMF preserves the baseline response underlying each ion. A positive-coefficient filter always produces a peak whose apex amplitude is the sum of the actual peak amplitude plus the underlying baseline response. Such background baseline intensity can be due to a combination of detector noise as well as other low-level peaks, sometimes termed chemical noise.

[0189] To obtain a more accurate measure of amplitude, a baseline subtraction operation is typically employed. Such an operation typically requires a separate algorithm to detect the baseline responses surrounding the peak, interpolate those responses to the peak center, and subtract that

response from the peak value to obtain the optimal estimate of the peak intensity.

[0190] Alternately, the baseline subtraction can be accomplished by specifying filters that have negative as well as positive coefficients. Such filters are sometimes referred to as deconvolution filters, and are implemented by filter coefficients that are similar in shape to filters that extract the second derivatives of data. Such filters can be configured to produce a single local-maximum response for each detected ion. Another advantage of such filters is that they provide a measure of deconvolution, or resolution enhancement. Thus, not only do such filters preserve the apex of peaks that appear in the original data matrix, but they can also produce apices for peaks that are visible only as shoulders, not as independent apices, in the original data. Consequently, deconvolution filters can address problems associated with co-elution and interference.

[0191] A third disadvantage of the GMF is that it generally requires a large number of multiplications to compute each data point in the output convolved matrix. Thus, convolution using a GMF is typically more computationally expensive and slower than convolution using other filters. As described below, filter specifications other than the GMF can be used in embodiments of the present invention.

Advantages of Second derivative filters

[0192] Filters that extract the second derivative of a signal are of particular use in detecting ions according to embodiments of the present invention. This is because the second derivative of a signal is a measure of the signal's curvature, which is the most prominent characteristic of a peak. Whether considered in one or two dimensions, a peak's apex is the point of the peak that has the highest magnitude of curvature. Shouldered peaks are also represented by regions of high curvature. Consequently, because of their responsiveness to curvature, second derivative filters can be used to enhance peak detection as well as provide improved detection for the presence of a shouldered peak against the background of a larger, interfering peak.

[0193] The second derivative at the apex of a peak has a negative value, because the curvature of a peak at its apex is maximally negative.

Embodiments of the present invention will use inverted second derivative filters. Inverted second derivative filter are second derivative filters all of whose coefficients have been multiplied by -1. The output of an inverted second derivative filters is positive at a peak apex. Unless otherwise specified, all second derivative filters referred to in the present invention are taken to be inverted second derivative filters. All plots of second derivative filters are inverted second derivative filters.

[0194] The response of a second derivative filter to a constant or straight line (having zero curvature) is zero. Thus the second derivative filter has zero response to the baseline response underlying a peak. The second derivative filter responds to the curvature at the apex of the peak and not to the underlying baseline. Thus the second derivative filter carries out, in effect, the baseline subtraction. Figure 15 illustrates the cross section of an exemplary second derivative filter that can be applied in either or both the chromatographic and spectral directions.

Second derivative filters in one-dimension

[0195] In a one-dimensional case, a second derivative filter is advantageous over a smoothing filter because the amplitude of the second derivative filter at the apex is proportional to the amplitude of the underlying peak. Moreover, the second derivative of the peak does not respond to the baseline. Thus, in effect, a second derivative filter performs the operation of baseline subtraction and correction automatically.

[0196] A disadvantage of second derivative filters is that they can have the undesirable effect of increasing noise relative to the peak apex. This noise-increasing effect can be mitigated by pre-smoothing the data or increasing the width of a second-derivative filter. For example, in one embodiment of the present invention, the width of the second-derivative convolution filter is increased. Increasing the width of the second-derivative convolution filter improves its ability to smooth the data in the input data matrix during convolution.

Savitzky-Golay filters for smoothing and obtaining a second-derivative

[0197] For a single-channel of data (spectrum or chromatogram), a conventional method for smoothing data (i.e., reducing the effects of noise)

or for differentiating data is through the application of a filter. In an embodiment of the present invention, smoothing or differentiating is performed on a one-dimensional data array by convolving that data array corresponding to the single spectrum or chromatogram with a set of fixed-value filter coefficients.

[0198] For example, well-known finite impulse response (FIR) filters can be specified with appropriate coefficients to perform a variety of operations including those of smoothing and differentiation. See example, KARL. Suitable smoothing filters generally have a symmetric, bell shaped curve, with all positive values, and a single maximum. Exemplary smoothing filters that can be used include filters having Gaussian, triangular, parabolic, trapezoidal shapes and co-sinusoidal shapes, each of which is characterized as a shape having a single maximum. Smoothing filters having asymmetric, tailed curves can also be used in embodiments of the present invention.

[0199] A family of FIR filters that can be specified to smooth or differentiate one-dimensional arrays of data is the well-known Savitzky-Golay filters. See example, in A. SAVITZKY & M.J.E. GOLAY, ANALYTICAL CHEMISTRY, VOL. 36, PP. 1627-1639 which is hereby incorporated by reference herein. The Savitzky-Golay (SG) polynomial filters provides a suitable family of smoothing and differentiating filters that are specified by sums of weighted polynomial shapes. The 0th order smoothing filter in this family of filters is a flat top (boxcar) filter. The second order smoothing filter in this family of filters is a parabola that has a single, positive maximum. The second order filter that obtains a second derivative in this family of filters is a parabola that has a single, negative maximum with zero mean. The corresponding inverted second derivative SG filter has a positive maximum.

Apodized Savitsky-Golay Filters

[0200] A modification of SG filters yields a class of smoothing and second derivative filters that work well in the present invention. These modified SG filters are known as Apodized Savitsky-Golay (ASG) filters. The term apodization refers to filter coefficients that are obtained by applying an array of weight coefficients to a least-squares derivation of SG filter

coefficients. The weight coefficients are the apodization function. For the ASG filter used in embodiments of the present invention, the apodization function is a cosine window (defined by COSINEWINDOW) in the software code below. This apodization function is applied, via weighted least-squares to a box-car filter to obtain the ASG smoothing filter, and to a second derivative SG quadratic polynomial, to obtain the ASG second derivative filter. The box car filter and second derivative quadratic are, by themselves, examples of Savitzky-Golay polynomial filters.

[0201] Every SG filter has a corresponding Apodized Savitzky-Golay (ASG) filter. An ASG filter provides the same basic filter function as the corresponding SG filter, but with higher attenuation of unwanted high-frequency noise components. Apodization preserves the smoothing and differentiation properties of SG filters, while producing much improved high-frequency cutoff characteristics. Specifically, apodization removes sharp transitions of the SG filter coefficients at the filter boundaries, and replaces them with smooth transitions to zero. (It is the cosine apodization function that forces the smooth transition to zero.). Smooth tails are advantageous because they reduce the risk of double counting due to high-frequency noise described above. Examples of such ASG filters include cosine smoothing filters and cosine-apodized second order polynomial Savitzky-Golay second derivative filters.

[0202] In the preferred embodiment of the present invention, these smoothing and second derivative ASG filters are specified for application to the column and rows of the LC/MS data matrix.

[0203] The following ANSI-C code returns the N filter coefficients of an Apodized Savitzky-Golay filters (ASG). The calling function (defined in the code below) is:

```
int ApodQuadFilterCoef (double *coef, int ncoef, int
nderiv) .
```

The number of coefficients, N, is supplied in the "ncoef" parameter. If the parameter "nderiv" is zero (0), the coefficients (returned in the array coef) are smoothing coefficients for an ASG filter. If the parameter "nderiv" is 2,

the coefficients (returned in the array coef) are second derivative coefficients from an ASG filter.

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*

TITLE: ApodQuadFilterCoef

PURPOSE: Returns Apodized Savitzky Golay filter coefficients for a quadratic polynomial model. The coefficients can extract from data a smoothed, first or second derivatives curve.

OPERATION: Coefficients are calculated from normal equations.

Design matrix for ncoef = 7 is

1	-3	9/2
1	-2	4/2
1	-1	1/2
1	0	0
1	1	1/2
1	2	4/2
1	3	9/2

Apodization is performed by a cosine window where

weight = [1+ cos(pi * ii/(half + 1))] / 2

so for ii=0, weight = 1

for ii=+/(half+1), weight = 0;

INPUT: coef pointer to array to which filter coefficients are written. User must allocate memory.

ncoef the number of coefficients, which must be an odd number >= 3.

nderiv = 0 smooth, = 1 first derivative, or
= 2 for second derivative.

RETURNS: ncoef Success. ncoef = the number of coef in coef.

-1 Failure, which occurs if ncoef <3,
or if nderiv is not equal to 0,1, or 2.

HISTORY: June 1998, M. Gorenstein

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```

/
#define COSINEWINDOW(kk,nhalf) ( 1.0+cos(PI * (double)kk/(nhalf+1.0))
)

```

```

int ApodQuadFilterCoef (double *coef, int ncoef, int nderiv)
{

```

```

    int ii, nhalf;
    double c00=0.0, c11=0.0, c22=0.0, c02=0.0, det;
    double d0, d1, d2, weight;

```

```

    nhalf = (ncoef-1)/2;
    ncoef = nhalf*2+1; /* Just in case ncoef is even */

```

```

    if (ncoef<3) return(-1);

```

```

    /* Computation is complicated by c02 cross term */

```

```

    if (nderiv==0 || nderiv ==2 )

```

```

    {
        /* Elements of correlation matrix */
        for (ii=-nhalf; ii<=nhalf; ii++)
        {
            weight = COSINEWINDOW(ii,nhalf);
            d0      = 1.0;
            d2      = ii*ii/2.0;
            c00    += SQR(weight)*d0*d0;
            c02    += SQR(weight)*d0*d2;
            c22    += SQR(weight)*d2*d2;
        }

```

```

    det = c00*c22 - SQR(c02);

```

```

    /* 2 by 2 matrix inversion performed in each expression */

```

```

    for (ii = -nhalf; ii<=nhalf; ii++)

```

```

    {
        weight = COSINEWINDOW(ii,nhalf);
        if (nderiv==0)
            coef[nhalf+ii] = SQR(weight)*(c22 - SQR(ii)*c02/2.0)/det;
        else
            coef[nhalf+ii] = SQR(weight)*(c00*SQR(ii)/2.0 - c02)/det;
    }

```

```

    }

    return(ncoef);
}
else if (nderiv==1)
{
    for (ii=1; ii<=nhalf; ii++)
    {
        weight = COSINEWINDOW(ii,nhalf);
        d1      = ii;
        c11     += SQR(weight)*d1*d1;
    }
    c11 *= 2.0;

    for (ii= -nhalf; ii<=nhalf; ii++)
    {
        weight = COSINEWINDOW(ii,nhalf);
        coef[nhalf+ii] = SQR(weight)* ii / c11;
    }

    return(ncoef);
}
/* Illegal derivative number */
return(-1);
}

```

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Example of Rank-1 filter for two-dimensional convolution

[0204] As an example of the application of a rank-1 formulation for two-dimensional convolution, we could choose f_p and g_q in Eq. (3) to have Gaussian profiles. The resulting F_{pq} has a Gaussian profile in each row and column. The values for F_{pq} will be close, but not identical to $f_{p,q}$ for the two-dimensional GMF. Thus, this particular rank-1 formulation will perform similarly to the GMF, but with a reduction in computation time. For example, in the example provided above, for example, where P and Q were equal to 20, computational load by using the rank-1 filter computational requirements reduced by a factor of $400/40 = 10$.

- [0205] The choice of f_p and g_q to have Gaussian profiles and the application of these filters according to Eq. (3) constitutes one embodiment of Step 2 and Step 3 according to the present invention.
- [0206] But for other embodiments of the present invention, we can apply separate filters for each dimension of a rank-1 filter. In an embodiment of the present invention, for example, f_p (the filter applied in the spectral direction) is a smoothing filter and g_q (the filter applied in the chromatographic direction) is a second derivative filter. Through such filter combinations, different rank-1 filter implementations can be specified that overcome problems typically associated with filtering. For example, the filters comprising a rank-1 filter can be specified to address the aforementioned problems associated with GMFs.
- [0207] The aforementioned rank-1 filters, implemented by Eq. 3 are more computationally efficient and therefore faster than the GMF implemented by Eq. 2. Moreover, the specified combination of filters provides a linear, baseline corrected response that can be used for quantitative work.
- [0208] Furthermore, the combination of filters sharpens, or partially deconvolves fused peaks in the chromatographic direction.
- [0209] An exemplary rank-1 filter for use in embodiments of the present invention that has the aforementioned advantages comprises a first filter, f_p , that is a co-sinusoidal ASG smoothing filter, whose FWHM is about 70% of the FWHM of the corresponding mass peak and a second filter, g_q , that is an ASG second-derivative filter, whose zero crossing width is about 70% of the FWHM of the corresponding chromatographic peak. Other filters and combinations of filters can be used as the rank-1 filters in other embodiments of the present invention.
- [0210] Figure 16A illustrates a cross section in the spectral direction of an exemplary co-sinusoidal ASG smoothing filter for use in a rank-1 filter to apply to the columns of the LC/MS data matrix to form an intermediate matrix. Figure 16B illustrates a cross section in the chromatographic direction of an exemplary, ASG second derivative filter to apply to the rows of the generated intermediate matrix.

[0211] The filter functions of f_p and g_q can be reversed. That is, f_p can be the second derivative filter and g_q can be the smoothing filter. Such a rank-1 filter deconvolves shouldered peaks in the spectral direction, and smoothes in the chromatographic direction.

[0212] Note that both f_p and g_q should not be second derivative filters. The rank-1 product matrix resulting where both f_p and g_q are second derivative filters contains not one, but a total of five, positive local maxima when convolved with an ion peak. The four additional positive apices are side-lobes that arise from the products of the negative lobes associated with these filters. Thus, this particular combination of filters results in a rank-1 filter that is not suitable for the proposed method.

[0213] The rank-2 formulation described below implements a filter that has properties of smoothing filters and second-derivative filters in both the spectral and chromatographic directions.

[0214] Several filter combinations for embodiments of the present invention that use a rank-1 convolution filters are provided Table 2.

m/z	Time
Smoothing	Smoothing
Smoothing	2 nd derive
2 nd derive	Smoothing

Table 2: Filter combinations for rank-1 filter

Each filter combination is an embodiment of Step 2, and each, being a rank-1 filter, is applied using Eq. (3), thereby embodying Step 3. Other filters and combinations of filters can be used as the rank-1 filters in other embodiments of the present invention.

Example of Rank-2 filter for two-dimensional convolution, which is the Preferred embodiment

[0215] The rank-2 filter requires specification of two filters for each of two dimensions. In a preferred embodiment of the present invention, the four filters are specified to address the problems associated with the GMF as described above in a computationally efficient manner.

[0216] For example, in an embodiment of the present invention, the first rank-1 filter comprises a spectral smoothing filter as f_p^1 and a chromatographic second derivative filter as g_q^1 . An exemplary such smoothing filter is a co-sinusoidal filter, whose FWHM is about 70% of the FWHM of the corresponding mass peak. An exemplary such second-derivative filter is ASG second-derivative filter, whose zero crossing width is about 70% of the FWHM of the corresponding chromatographic peak. The second rank-1 filter comprises a spectral second derivative filter as f_p^2 and a chromatographic smoothing filter as g_q^2 . An exemplary such second derivative filter is a second derivative ASG filter, whose zero-crossing width is about 70% of the FWHM of the corresponding mass peak. An exemplary such smoothing filter is a co-sinusoidal filter, whose FWHM is about 70% of the FWHM of the corresponding chromatographic peak. Other filters and filter combinations can be used in embodiments of the present invention. The cross sections of such filters are illustrated in Figures 16C, 16D, 16E, and 16F respectively.

[0217] The rank-2 filter described above has several advantages over the GMF. Because it is a rank-2 filter, it is more computationally efficient than the GMF and consequently faster in execution. Moreover, because each cross-section is a second derivative filter whose coefficients sum to zero, it provides a linear, baseline corrected response that can be used for quantitative work and it sharpens, or partially deconvolves, fused peaks in the chromatographic and spectral directions.

[0218] In a preferred rank-2 filter embodiment of the present invention, the filter widths of each of the column filters (in terms of number of coefficients) are set in proportion to the spectral peak width and the filter widths of each of the row filters (in terms of number of coefficients) are set in proportion to the chromatographic peak width. In the preferred embodiment of the present invention, the widths of the column filters are set equal to each other, and in proportion to the FWHM of a spectral peak. For example, for a spectral peak width FWHM of 5 channels, the filter width may be set to 11 points, so the filter width of both the smoothing and second

derivative spectral filter will be set to the same value of 11 points. Analogously, in the preferred embodiment, the widths of the row filters are set equal to each other, and in proportion to the FWHM of a chromatographic peak. For example, for a chromatographic peak width FWHM of 5 channels, the filter width can be set to 11 points, so the filter width of both the smoothing and second derivative spectral filters will be set to the same value of 11 points. Choosing the filter widths in this manner results in rank-1 filters comprising the rank-2 filter having equal dimensions. That is, if the first rank-1 filter has dimension M X N, then the dimension of the second rank-1 filter also has dimension M X N. It should be noted that the rank-2 filter need not be comprised of rank-1 filters having equal dimensions and that any suitable rank-1 filters can be summed to produce a rank-2 filter.

[0219] The rank-1 filters are summed to construct the rank-2 filter, therefore the filters must be normalized in a relative sense prior to summing. In the preferred embodiment, the first rank-1 filter is a smoothing filter in the spectral direction and is a second derivative filter in the chromatographic direction. If this filter is weighted more than the second rank-1 filter, then the combined filter gives more emphasis to smoothing in the spectral direction and baseline-subtraction and deconvolution of peaks in the chromatographic direction. Thus the relative normalization of the two rank-1 filters determines the relative emphasis of smoothing and differentiation in the chromatographic and spectral directions.

[0220] For example, consider two rank-1 filters:

$$F_{p,q}^1 = f_p^1 g_q^1 \quad (11)$$

$$F_{p,q}^2 = f_p^2 g_q^2 \quad (12)$$

where, equation (11) is the first rank-1 filter, and equation (12) is the second rank-1 filter. In a preferred embodiment of the present invention, each rank-1 filter is normalized so that the sum of its coefficients squared equals one. This normalization gives equal weight for smoothing and differentiation to the spectral and chromatographic directions. That is, for rank-1 filters, each having dimensions of M x N:

$$\sum_{q=1}^N \sum_{p=1}^M (f_p^1 g_q^1)^2 = 1$$

$$\sum_{q=1}^N \sum_{p=1}^M (f_p^2 g_q^2)^2 = 1.$$

[0221] The smoothing filters and second derivative filters of the preferred embodiments can be normalized to satisfy this criterion by applying an appropriate scaling factor to the coefficients of the respective rank-1 matrices.

[0222] Moreover, in the preferred embodiment, the row dimension of each rank-1 filter is the same, and the column dimensions of each rank-1 filter is the same. As a result, the coefficients can be added to obtain the rank-2 convolution filter's point source as follows:

$$F_{p,q} = f_p^1 g_q^1 + f_p^2 g_q^2 \quad (13)$$

From equation (13), it can be seen that the relative normalization of the two rank-1 filters is needed to determine the two-dimensional convolution filter $F_{p,q}$.

Filter coefficients for preferred embodiment of two-dimensional convolution filter

[0223] An exemplary rank-2 filter is described with respect to Figures 17A-K. This filter is an embodiment of Step 2 and Step 3 that can be used to detect ions, subtract baseline response, resolved partially fused peaks, and perform with high computational efficiency.

[0224] In particular, this rank-2 filter is useful for detecting shouldered peaks. A rank-2 filter according to embodiments of the present invention can comprise a second derivative filter in both the chromatographic and spectral directions. Due to the responsive nature of second derivatives filters to curvature, such a rank-2 filter can detect shouldered peaks wherein the apex of the shouldered peak may not be evident in the data. Given that the rank-2 filter comprises a second derivative filter, which measures

curvature, the apex of the second peak, which is not seen in the data directly, can be detected as a separate apex in the output convolved matrix.

[0225] Figure 17A is a graphical representation of a simulated peak that can be generated in LC/MS data, wherein the horizontal axes represent time of scan and m/z channel as shown, and the vertical axis represents intensity. Figure 17B illustrates the convolution filter matrix corresponding to the rank-2 filter, according to a preferred embodiment of the present invention.

[0226] In this simulation, the spectral and chromatographic peak widths of all ions are 8 points, FWHM. The number of filter coefficients for all four filters is 15 points.

[0227] Figure 17C illustrates a simulation of two LC/MS peaks that have the same mass, and are nearly, but not identically, co-incident in time. Figure 17D illustrates that the peak cross section is a pure peak in mass, and Figure 17E illustrates that the peak cross section exhibits a shoulder 1704 in time. Figures 17F through 17H illustrate the effect of simulated counting (shot noise) on each sampled element comprising the shouldered peak illustrated in Figures 17D-17E. Figure 17G and 17H illustrate the cross sections arising due to the added counting noise. As can be seen in both Figures 17G and 17H, many local maxima are generated as a result of the counting noise. Thus, it can be seen that even though only two ions are present, counting noise can produce numerous spurious local maxima that could cause false positive ion detection.

[0228] Figures 17I-K illustrates the results of convolving a rank-2 filter with the simulated data. The resultant output convolved matrix (represented by the contour plot of Figure 17I) comprises two distinct peaks 1702 and 1706. Peak 1702 is the peak associated with the more intense of the two ions, and peak 1706 is the peak of the less intense shouldered ion. Figure 17J is a cross section of the output convolved matrix in the spectral (mass-to-charge ratio) direction. Figure 17K is a cross section of the output convolved matrix in the chromatographic (time) direction.

[0229] What is observed by reviewing Figures 17I-K is that a rank-2 filter-based embodiment of the present invention reduces the effect of counting noise and deconvolutes shouldered peaks to produce multiple local maxima.

Each local maximum is associated with an ion. As a result, this embodiment of the present invention also reduces the false positives rate. The ion parameters, m/z , retention time and intensity are obtained by analyzing the detected local maxima as described above.

Any filter can be used if it produces a single local maximum

[0230] The filters and convolution methods described above can be used to detect ions in an LC/MS data matrix. Other sets of filter coefficients can be chosen as embodiments of Step 2.

[0231] The input signal is a peak in the LC/MS data matrix that has a unique maximum, so the convolution filter of Step 2 must faithfully maintain that unique positive maximum through the convolution process. The general requirement that a convolution filter must satisfy for it to be an embodiment of Step 2 is as follows: the convolution filter must have an output that produces a unique maximum when convolved with an input having a unique maximum.

[0232] For an ion that has a bell-shaped response, this condition is satisfied by any convolution filter whose cross sections are all bell-shaped, with a single positive maximum. Examples of such filters include inverted parabolic filters, triangle filters, and co-sinusoidal filters. Specifically, any convolution filter that has the property that it has a unique, positive valued apex makes that filter a suitable candidate for use in embodiments of the present invention. A contour plot of the filter coefficients can be used to examine the number and location of the local maxima. All row, column and diagonal cross sections through the filter must have a single, positive, local maximum. Numerous filter shapes meet this condition and can therefore be employed in embodiments of the present invention.

Boxcar can be used because it produces a single local maximum

[0233] Another filter shape that is acceptable is a filter having a constant value (*i.e.*, a boxcar filter). This is because convolution of a peak with a boxcar filter produces an output that has a single maximum. A well-known characteristic of boxcar filters that is advantageous in embodiments of the present invention is that such a shape produces minimum variance for a

given number of filter points. Another advantage of boxcar filters is that in general they can be implemented with fewer multiplications than filters having other shapes such as Gaussian or co-sinusoidal filters.

[0234] The dimensions of the boxcar should match the extent of the peak in both the spectral and chromatographic directions. If the boxcar is too small, not all counts associated with a peak will be summed. If the boxcar is too large, then counts from other, neighboring peaks may be included.

[0235] However, boxcar filters also have distinct disadvantages for some applications to which the present invention might be applied. For example, the transfer function of boxcar filters reveals that they pass high frequency noise. Such noise can increase the risk of double counting peaks for low amplitude signals (low SNR), which might be undesirable in some applications of the present invention. Consequently, filter shapes other than boxcar shapes are generally preferred in applications of the present invention.

Second derivative filters can produce a single local maximum

[0236] Another suitable class of convolution filters that have an output that produces a unique maximum when convolved with an input having a unique maximum are filters that have a single, positive local maximum, but have negative side-lobes. Examples of such filters include second derivative filters, which are responsive to curvature. A suitable second derivative filter can be specified by subtracting the mean from a smoothing filter. Though such filters can be assembled from combinations of boxcar, triangular and trapezoidal shapes, the most common specification of filters that differentiate data are Savitzky-Golay polynomial filters.

Gaussian noise and Poisson noise

[0237] The Gaussian Matched Filter is an optimum filter when the noise has Gaussian statistics. The noise from counting detectors has Poisson statistics. In the case of Poisson noise the boxcar filter may be an optimal filter for use in detection because the boxcar simply sums all counts associated with a peak. However, many of the limitations described for GMF still apply to the boxcar filter, even in the case of Poisson noise. The boxcar filter cannot subtract baseline noise and cannot resolve interfered and coeluted peaks. In

addition, the transfer function of the boxcar filter may allow for double counting for peak apices.

[0238] The rank-2 filter of the preferred embodiments is a compromise in SNR for both the case of Gaussian noise and Poisson noise. This rank-2 filter has the advantage of baseline subtraction and partial resolution of overlapped peaks.

Role of peak width in determining filter coefficients

[0239] In embodiments of the present invention, the coefficients of the convolution filter F to be convolved with the input matrix D are chosen to correspond to the typical shape and width of a peak corresponding to an ion. For example, the cross section of the central row of filter F matches the chromatographic peak shape; the cross section of the central column of filter F matches the spectral peak shape. It should be noted that although the width of the convolution filter can be matched to the FWHM of the peak (in time and in mass-to-charge), such width matching is not required.

Interpretation of ion intensity and scaling of filter coefficients

[0240] In the present invention, the intensity measurement estimate is the response of the filter output at the local maximum. The set of filter coefficients with which the LC/MS data matrix is convolved determines the scaling of the intensity. Different sets of filter coefficients result in different intensity scalings, so this estimate of intensity of the present invention does not necessarily correspond exactly to peak area or peak height.

[0241] However, the intensity measurement is proportioned to peak area or to peak height since the convolution operation is a linear combination of intensity measurements. Thus the response of the filter output at local maximum is in proportion to the molecule's concentration in the sample that gave rise to the ion. The response of the filter output at local maximum can then be used for the purpose of quantitative measurement of molecules in the sample in the same way as the area or height of the peak of the ion's response.

[0242] Provided a consistent set of filters is used to determine the intensities of standards, calibrators and sample, the resulting intensity measurements produce accurate, quantifiable results regardless of the intensity scaling. For

example, intensities generated by embodiments of the present invention can be used to establish concentration calibration curves which can thereafter be used to determine the concentration of analytes.

Asymmetric peak shapes

[0243]

The examples above have assumed that an ion's peak shapes in the spectral and in the chromatographic directions are Gaussians, and therefore symmetric. In general, peak shapes are not symmetric. A common example of an asymmetric peak shape is that of a tailed Gaussian; a Gaussian convolved with a step-exponential. The methods described here still apply to peak shapes that are asymmetric. In the case where a symmetric filter is applied to an asymmetric peak, the location of the apex in the output convolved matrix will not, in general, correspond exactly to the apex location of the asymmetric peak. However, any offset originating from peak asymmetry (in either the chromatographic or the spectral direction) will be, effectively a constant offset. Such an offset is easily corrected for by conventional mass spectrometric calibration, and by retention time calibration using an internal standard.

[0244]

According to The Matched Filter Theorem, the optimum shape for detection for an asymmetric peak will be the asymmetric peak shape itself. However, provided the width of the symmetric filter matches the width of the asymmetric peak, the difference in detection efficiency between a symmetric filter and a matched asymmetric filter will be minimal for the purposes of this invention.

Changing filter coefficients to interpolate and offset data

[0245]

Another use of coefficient modification is to provide interpolation to account for small changes due to calibration of the mass spectrometer. Such coefficient modification can occur from spectrum to spectrum. For example, if a change in mass calibration causes an offset of a fraction of a channel by 0.3, then column filters (both smoothed and second derivative) can be derived that estimate what the output would be in the absence of such a mass offset. In this manner, a real-time mass correction can be made. Typically, the resulting filter is slightly asymmetric.

Dynamic Filtering

[0246]

Filter characteristics such as the filter width scaling can be changed in response to known changing characteristics of the LC separation or of the MS scans. For example, in a time of flight (TOF) mass spectrometer, the peak width (FWHM) is known to change from low values (such as 0.010 amu) to wider values (such as 0.130 amu) over the course of each scan. In a preferred embodiment of the present invention, the number of coefficients of the smoothing and differentiating filters is set equal to approximately twice the FWHM of the spectral peak. As the MS scan progresses, for example, from low to high mass, the filter width of both the smoothing and second derivative column filters employed by the preferred embodiment can be expanded accordingly to preserve the relationship between filter width and peak width. Analogously, if the width of the chromatographic peak is known to change during a separation, the width of the row filters can be expanded or contracted to preserve the relationship between filter width and peak width.

Real-Time Embodiments of rank-1 and rank-2 filters

[0247]

In conventional LC/MS systems, spectra are acquired as the separation progresses. Typically spectra are written to computer memory at a constant sample rate (*e.g.*, at a rate of once per second). After one or more complete spectra are collected, they are written to more permanent storage, such as to a hard disk memory. Such post collection processing can be performed in embodiments of the present invention as well. Thus, in one embodiment of the present invention, the convolution matrix is generated only after the acquisition is complete. In such an embodiment of the present invention, the original data and the convolved matrix itself are stored as is the ion parameter list obtained from analyzing the detected local maxima.

[0248]

In addition, embodiments of the present invention using rank-1 and rank-2 filters can be configured to operate in real time. In a real-time embodiment of the present invention, the columns of the convolution matrix are obtained while the data is being acquired. Thus, the initial columns (corresponding to spectra) can be obtained, analyzed, and have their ion parameters written to disk before the acquisition of all spectra is complete.

[0249] This real-time embodiment of the present invention essentially analyzes the data in computer memory, writing only the ion parameter list to the permanent hard disk drive. In this context, real time means that rank-1 and rank-2 filtering is performed on the spectra in computer memory as the data is being acquired. Thus, ions detected by the LC/MS in the beginning of the separation are detected in the spectra written to disk, and the portion of the ion parameter list containing parameters associated with these ions is also written to disk as the separation proceeds.

[0250] There is typically a time delay associated with beginning real-time processing. The spectra that contain ions that elute in a chromatographic peak at time, t , and width, Δt , can be processed as soon as they are collected. Typically, real-time processing begins at time $t + 3\Delta t$, i.e., after 3 spectra are initially collected. Ion parameters determined by analysis of this chromatographic peak are then appended to an ion parameters list being created and stored in permanent storage, such as a computer disk. The real-time processing proceeds according to the techniques described above.

[0251] Advantages of real-time processing include: (1) quick acquisition of the ion parameter list; (2) triggering real-time processes based upon information in the ion parameter list. Such real-time processes include fraction collection and stop flow techniques to store eluent for analysis. An exemplary such stop-flow technique traps the eluent in a nuclear-magnetic-resonance (NMR) spectral detector.

[0252] Figure 18 is a flow chart 1800 illustrating a method for real-time processing according to a preferred embodiment of the present invention. The method can be executed in hardware, for example, for example in a DSP-based design or in software, such as in the DAS described above. It would be apparent to those skilled in the art how to configure such hardware or software based on the following description. For ease of description, the method is described as if performed by the DAS executing software. Figure 19 illustrates a spectral buffer 1902, chromatographic buffer 1906 and an apex buffer 1910 and how they are manipulated in performing the method illustrated in flow chart 1800.

[0253] The DAS begins the method in step 1802 by receiving the next spectral element. In Figure 19, these spectral elements are shown as S1, S2, S3, S4 and S5 and correspond to spectral elements received at times T1, T2, T3, T4 and T5 respectively. In step 1804, the DAS determines whether the received spectral element is 0 or not. If the received spectral element is 0, the DAS continues the method in step 1802 by receiving the next spectral element. If the spectral element is not zero, its intensity is used to scale the coefficients of a spectral filter 1904. In the example illustrated in Figure 19, spectral filter 1904 is a 3-element filter having filter coefficients F1, F2 and F3. The scaling can be accomplished by multiplying each filter coefficient by the intensity of the received spectral element.

[0254] In step 1808, the scaled spectral filter coefficients are added to a spectral buffer. The spectral buffer is an array. The number of elements in the spectral buffer equals the number of elements in each spectrum.

[0255] For performing the summation, filter 1904 is aligned so that the element of the spectral buffer corresponding to the received spectral element is aligned with the center of filter 1904. Thus, at time T1, when spectral element S1 is received, the center of filter 1904, F2, is aligned with spectral buffer element 1902a; at time T2, when spectral element S2 is received, the center of filter 1904, F2, is aligned with spectral buffer element 1902b, and so on. These steps are illustrated in Figure 19, wherein the scaling of filter coefficients F1, F2, and F3 and addition to spectral buffer 1902 is illustrated for times, T1, T2, T3, T4 and T5, which, in the present example, is the time required to receive sufficient spectral elements to fill spectral buffer 1902. The resulting scaled sums are also shown in the spectral buffer elements of Figure 19.

[0256] In step 1810, the DAS determines whether the spectral buffer is full, that is, whether the number of spectral elements received and processed is the same as the number of elements in the spectral filter. If not, then the DAS continues the method in step 1802 by waiting for the next spectral element. If the spectral buffer is full, the DAS continues the method in step 1812.

[0257] In step 1812, the DAS moves the new spectrum to chromatographic buffer 1906. Chromatographic buffer 1906 contains N-spectra, where N is the number of coefficients in the chromatographic buffer. In the present example, N is 3. Chromatographic buffer 1906 is configured as a first in, last out (FILO) buffer. Consequently, when a new spectrum is added, the oldest spectrum is dropped. When a new spectrum is added in step 1812, the oldest spectrum is discarded. In step 1814, the DAS applies a chromatographic filter 1907 to each row of chromatographic buffer 1906. After application of the filter, central column 1908 corresponds to a single column convolved spectrum of the output convolved matrix. In step 1816, the DAS transfers the convolved spectrum to an apex buffer 1910.

[0258] In an embodiment of the present invention, apex buffer 1910 is three spectra in width, that is, apex buffer 1910 comprises three columnar spectra. Each of the spectral columns preferably has the length of a complete spectrum. Apex buffer 1910 is a FILO buffer. Thus, when the new column from chromatographic buffer 1906 is appended to apex buffer 1910 in step 1816, the oldest columnar spectrum is discarded.

[0259] Peak detection algorithms as described below can be performed on the central column 1912 of apex buffer 1910. Central column 1912 is used to provide more accurate analysis of peaks and ion parameters by using nearest neighbor values. Analysis of the peaks allows the DAS to extract ion parameters (such as retention time, m/z and intensity) in step 1820 to store in the ion parameter list. Moreover, spectral peak width information can also be obtained by examining points adjacent to the local maxima along the column.

[0260] Apex buffer 1910 can also be expanded beyond 3 spectra in width. For example, to measure chromatographic peak width, it would be necessary to expand the apex buffer to include a number of spectra at least equal to the FWHM of the chromatographic peak, for example twice the FWHM of a chromatographic peak.

[0261] In a real-time embodiment of the present invention, original spectra need not be recorded. Only the filtered spectra are recorded. Thus, the mass storage requirements for a real time embodiment of the present

invention are reduced. Generally, however, additional storage memory, for example, RAM, is required for real-time embodiments of the present invention. For a rank-1 filter-based real time embodiment of the present invention, only a single spectrum buffer is needed. For rank-2 filter-based real time embodiment of the present invention, two spectral buffers are needed, one for the smoothing, and one for the second derivative spectral filters.

STEP 4: Peak Detection

[0262] The presence of an ion produces a peak having a local maximum of intensity in the output convolved matrix. The detection process of embodiments of the present invention detects such peaks. In one embodiment of the present invention, the detection process identifies those peaks whose maximal intensity satisfy a detection threshold as peaks that correspond to ions. As used herein, satisfaction of a detection threshold is defined as meeting any criterion for overcoming the detection threshold. For example, the criterion could be meeting the detection threshold or meeting or exceeding the detection threshold. In addition in some embodiments of the present invention, the criterion may be falling below a detection threshold or meeting or falling below a detection threshold.

[0263] Each local maximum of intensity in the output convolved matrix is a candidate for a peak that corresponds to an ion. As described above, in the absence of detector noise, every local maximum would be deemed to correspond to an ion. However, in the presence of noise, some local maxima (especially low-amplitude local maxima) are due only to the noise, and do not represent genuine peaks corresponding to detected ions. Consequently, it is important to set the detection threshold to make it highly unlikely that a local maximum that satisfies the detection threshold is due to noise.

[0264] Each ion produces a unique apex or maximum of intensity in the output convolved matrix. The characteristics of these unique maxima in the output convolved matrix provide information on the number and properties of the ions present in the sample. These characteristics include location, width and other properties of the peaks. In one embodiment of the present

invention, all local maxima in the output convolved matrix are identified. Subsequent processing eliminates those determined not to be associated with ions.

[0265] According to embodiments of the present invention, a local maximum of intensity is deemed to correspond to a detected ion only if the value of the local maximum satisfies a detection threshold. The detection threshold itself is an intensity against which local maxima of intensities are compared. The value of the detection threshold can be obtained by subjective or objective means. Effectively, the detection threshold divides the distribution of true peaks into two classes: those that satisfy the detection threshold and those that do not satisfy the detection threshold. Peaks that do not satisfy the detection threshold are ignored. Consequently, true peaks that do not satisfy the detection threshold are ignored. Such ignored true peaks are referred to as false negatives.

[0266] The threshold also divides the distribution of noise peaks into two classes: those which satisfy the detection threshold and those which do not satisfy the detection threshold. Any noise peaks that satisfy the detection threshold are deemed ions. Noise peaks that are deemed ions are referred to as false positives.

[0267] In embodiments of the present invention, the detection threshold typically is set to achieve a desired false positive rate, which is usually low. That is, the detection threshold is set so that the probability that a noise peak will satisfy the detection threshold in a given experiment is unlikely.

[0268] To obtain a lower false positive rate, the detection threshold is set to a higher value. Setting the detection threshold to a higher value to lower the false positive rate has the undesirable effect of raising the false negative rate, i.e., the probability that low-amplitude, true peaks corresponding to ions will not be detected. Thus, the detection threshold is set with these competing factors in mind.

[0269] The detection threshold can be determined subjectively or objectively. The goal of any thresholding method, whether subjective or objective is to determine a detection threshold to use to edit the ion list. All

peaks whose intensities do not satisfy the detection threshold are considered noise. These "noise" peaks are rejected and not included in further analysis.

[0270]

A subjective method for setting the detection threshold is to draw a line that is close to the maximum of the observed noise. Any local maxima satisfying the detection threshold are considered peaks corresponding to ions. And any local maxima not satisfying the detection threshold are considered noise. Although the subjective method for determining threshold can be used, objective techniques are preferred.

[0271]

One objective method for selecting the detection threshold according to embodiments of the present invention uses a histogram of the output convolved matrix data. Figure 20 is a flow chart for a method for objectively determining a detection threshold according to an embodiment of the present invention. The method is also graphically illustrated in Figure 7. The method proceeds according to the following steps:

STEP 2002: Sort the intensities of all positive local maxima found in the output convolved matrix in ascending order

STEP 2004: Determine standard deviation of intensity data in output convolved data matrix as the intensity that is at the 35.1 percentile in the list.

STEP 2006: Determine the detection threshold based on a multiple of the standard deviation.

STEP 2008: Generate edited ion list or ion parameter list using peaks satisfying the detection threshold.

[0272]

The above method is applicable when most of the local maxima are due to Gaussian noise. For example, if there 1000 intensities, then Step 2004 would determine that the 351th intensity represents a Gaussian standard deviation. If the distribution of maximal intensities were due only to Gaussian noise processes, then local maxima whose values exceeded the 351st intensity would occur at frequency that is predicted by a Gaussian noise distribution.

[0273]

The detection threshold is then a multiple of the 351th intensity. As an example, consider two detection thresholds. One corresponds to 2 standard deviations. One corresponds to 4 standard deviations. The 2-

deviation threshold yields few false negatives, but a large number of false positives. From the properties of a Gaussian noise distribution a 2-standard deviation threshold means that about 5% of peaks would be falsely identified as ions. The 4-deviation threshold yields more false negative, but significantly fewer false positives. From the properties of a Gaussian noise distribution a 4-standard deviation threshold means that about 0.01% of peaks would be falsely identified as ions.

[0274] Rather than sorting the list of intensities of all local maxima, a histogram display can be used where the number of intensities per interval of intensities are recorded. A histogram is obtained by selecting a series of uniformly spaced intensity values, each pair of values defining an interval, and counting the number of maximal intensities that fall within each bin. The histogram is the number of intensities per bin versus the mean intensity value defining each bin. The histogram provides a graphical method for determining the standard deviation of the distributions of intensities.

[0275] A variation of the empirical method uses the relationship between the standard deviation σ of the convolved output noise and the standard deviation σ_o of the input noise to set the detection threshold. From the filter

analysis above, this relationship is given as
$$\sigma = \sigma_o \sqrt{\sum_{j=-l}^l \sum_{i=-h}^h F_{i,j}^2}$$
 assuming that the input noise is uncorrelated Gaussian deviates. The input noise

σ_o can be measured from the input LCL/MS data matrix as the standard deviation of the background noise. A region of the LC/MS containing only background noise can be obtained from a blank injection, that is LC/MS data is obtained from a separation with no sample injected.

[0276] Thus, the standard deviation of the output can be inferred using only the values of the filter coefficients $F_{i,j}$ and the measured background noise σ_o . The detection threshold can then be set based upon the derived output noise standard deviation σ .

STEP 5: Peak Parameter extraction

[0277] After identifying those local maxima that are peaks corresponding to ions, parameters for each peak are estimated. In one embodiment of the present invention the parameters that are estimated are the retention time, mass-to-charge ratio, and intensity. Additional parameters such as chromatographic peak width (FWHM) and mass-to-charge peak width (FWHM) can also be estimated.

[0278] The parameters of each identified ion are obtained from the characteristics of the local maximum of the detected peaks in the output convolved data matrix. In an embodiment of the present invention, these parameters are determined as follows: (1) the ion's retention time is the time of the (filtered) scan containing the (filtered) maximal element (2) The ion's m/z is the m/z of the (filtered) channel containing the (filtered) maximal element; (3) The ion's intensity is the intensity of the (filtered) maximal element itself.

[0279] The width of a peak in the spectral or chromatographic directions can be determined by measuring the distance between the locations of the nearest zero crossing points that straddle the peak or by measuring the distance between the nearest minima that straddle a peak. Such peak widths can be used to confirm that a peak is resolved from its neighbors. Other information can be gathered by considering peak width. For example, an unexpectedly large value for a peak width may indicate coincident peaks. Consequently, the locations of zero crossings or local minima can be used as inputs to estimate the effect of interfering coincidence or to modify parameter values stored in the ion parameter list.

[0280] The parameters determined by analyzing the peaks can be further optimized by considering the neighboring elements. Because the elements of the convolved matrix represent a digital sample of data, the true apex of a peak in the chromatographic (time) dimension may not coincide exactly with a sample time and the true apex of a peak in the spectral (mass-to-charge ratio) dimension may not coincide exactly with a mass-to-charge ratio channel. As a result, typically the actual maximum of the signal in the time and mass-to-charge ratio dimensions is offset from the available sampled values by a fraction of the sample period or the mass-to-charge

ratio channel interval. These fractional offsets can be estimated from the values of the matrix elements surrounding the element having the local maximum corresponding to the peak using interpolation, such as curve fitting techniques.

[0281] For example, a technique for estimating the fractional offset of the true apex from an element of the output convolved matrix containing a local maxima corresponding to an ion in the two-dimensional context is to fit a two-dimensional shape to the elements of the data matrix containing a local maxima and its nearest neighbors. In embodiments of the present invention, a two-dimensional parabolic shape is used because it is a good approximation to the shape of the convolved peak near its apex. For example, a parabolic shape can be fit to a nine element matrix comprising the peak and its 8 nearest neighbors. Other fits can be used for this interpolation within the scope and spirit of the present invention.

[0282] Using the parabolic fit an interpolated value of the peak apex is calculated from which to determine the ion parameters. The interpolated value provides more accurate estimates of retention time, m/z and intensity than those obtained by reading values of scan times and spectral channels. The value of the parabola at the maximum, and its interpolated time and m/z values corresponding to that maximum, are the estimates of ion intensity, retention time and m/z .

[0283] The interpolated location in the row direction of the maximum of the two-dimensional parabolic fit is an optimal estimate of retention time. The interpolated location in the column direction of the maximum of the two-dimensional parabolic fit gives an optimum estimate of mass-to-charge ratio. The interpolated height of the apex above baseline gives an optimum estimate (scaled by filter factors) of ion intensity or concentration.

[0284] Embodiments of the present invention can also be configured to extract peak parameters from the results of intermediate convolved matrices. For example, the methods discussed above for locating a single peak corresponding to a detected ion can also be used to locate peaks in each row or column of the matrix. These peaks may be useful to store a spectra or chromatograms at known times or mass values.

[0285] For example, spectra or chromatograms obtained from the second derivative filters can be obtained for each row and column from the intermediate convolved matrices described above. These intermediate results can be examined for local maxima as well. These maxima are, in effect smoothed versions of the chromatograms and spectra. Local maxima can be extracted and saved, giving additional detail as to the spectral content of the sample at a particular time or time range, or the chromatographic content at a typical mass or mass range.

Measurement Error

[0286] Because each ion parameter measurement produced by embodiments of the present invention is an estimate, there is a measurement error associated with each such measurement. These associated measurement errors can be statistically estimated.

[0287] Two distinct factors contribute to the measurement errors. One factor is a systematic or calibration error. For example, if the mass spectrometer m/z axis is not perfectly calibrated, then any given m/z value contains an offset. Systematic errors typically remain constant. For example, calibration error remains essentially constant over the entire m/z range. Such an error is independent of signal-to-noise or amplitude of a particular ion. Similarly, in the case of mass-to-charge ratio, the error is independent of the peak width in the spectral direction.

[0288] The second factor contributing to measurement error is the irreducible statistical error associated with each measurement. This error arises due to thermal or shot-noise related effects. The magnitude or variance of this error for a given ion depends on the ion's peak width and intensity. Statistical errors measure reproducibility and therefore are independent of calibration error. Another term for the statistical error is precision.

[0289] The statistical error associated with each measurement can in principle be estimated from the fundamental operating parameters of the instrument on which the measurement is made. For example in a mass spectrometer, these operating parameters typically include the ionization and transfer efficiency of the instrument coupled with the efficiency of the

micro-channel counting plate (MCP). Together, these operating parameters determine the counts associated with an ion. The counts determine the statistical error associated with any measurement using the mass spectrometer. For example, the statistical error associated with the measurements discussed above typically follows a Poisson distribution. A numerical value for each error can be derived from counting statistics via the theory of error propagation. See example, in P.R. BEVINGTON, DATA REDUCTION AND ERROR ANALYSIS FOR THE PHYSICAL SCIENCES at 58-64 (MCGRAW-HILL 1969)

[0290] In general, statistical errors also can be inferred directly from the data. One way to infer statistical errors directly from the data is to investigate the reproducibility of the measurements. For example, replicate injections of the same mixture can establish the statistical reproducibility of m/z values for the same molecules. Differences in the m/z values through the injections are likely due to statistical errors.

[0291] In the case of errors associated with retention time measurements, statistical reproducibility is more difficult to achieve because systematic errors arising from replicate injections tend to mask the statistical error. A technique to overcome this difficulty is to examine ions at different m/z values that were produced from a common parent molecule. Ions that originate from a common molecule would be expected to have identical intrinsic retention times. As a result, any difference between measurements of the retention times of molecules originating from a common parent is likely due to statistical errors associated with the fundamental detector noise associated with measurements of peak properties.

[0292] Each measurement made and stored using an embodiment of the present invention can be accompanied by estimates of its associated statistical and systematic errors. Though these errors apply to the parameter estimates for each detected ion, their values can be inferred generally by analyzing sets of ions. After a suitable error analysis, the errors associated with each measurement for a detected ion can be included in each row of the table corresponding to the detected ion measurement. In such an embodiment of the present invention, each row of the table can have fifteen

measurements associated with each ion. These measurements are the five measurements for the detected ion corresponding to the row and their associated statistical and systematic errors, which are retention time, mass-to-charge ratio, intensity, spectral FWHM, and chromatographic FWHM.

[0293] As described above, the statistical component of measurement error, or precision, in retention time and m/z depends on the respective peak widths and intensities. For a peak that has a high SNR, the precision can be substantially less than the FWHM of the respective peak widths. For example, for a peak that has a FWHM of 20 milli-amu and high SNR, the precision can be less than 1 milli-amu. For a peak that is barely detectable above the noise, the precision can be 20 milli-amu. For purposes of the present discussion of statistical error, the FWHM is considered to be the FWHM of the peak in the LC/MS chromatogram prior to convolution.

[0294] Precision is proportional to the peak width and inversely proportional to peak amplitude. Generally, the relationship between precision, peak width and peak amplitude can be expressed as:

$$\sigma_m = k \frac{w_m}{h_p}$$

[0295] In this relationship, σ_m is the precision of the measurement of m/z (expressed as a standard error), w_m is the width of the peak (expressed in milli-amu at the FWHM), h_p is the intensity of the peak (expressed as a post-filtered, signal to noise ratio) and k is a dimensionless constant on the order of unity. The exact value for k depends on the filter method used. This expression shows that σ_m is less than w_m . Thus, the present invention allows estimates of m/z for a detected ion to be made with a precision that is less than the FWHM of the m/z peak width as measured in the original LC/MS data.

[0296] Similar considerations apply with respect to the measurement of retention time. The precision to which retention time of a peak can be measured depends on the combination of peak width and signal intensity. If the FWHM max of the peak is 0.5 minutes, the retention time can be measured to a precision, described by a standard error, of 0.05 minutes (3

seconds). Using the present invention, estimates of retention time for a detected ion can be made with a precision that is less than the FWHM of the retention time peak width as measure in the original LC/MS data.

STEP 6: Store extracted parameters

[0297] As described above, one output of embodiments of the present invention is a table or list of parameters corresponding to detected ions. This ion parameter table, or list has a row corresponding to each detected ion, wherein each row comprises one or more ion parameters and, if desired, their associated error parameters. In one embodiment of the present invention, each row in the ion parameter table has three such parameters: retention time, mass-to-charge ratio and intensity. Additional ion parameters and associated errors can be stored for each detected ion represented in the list. For example, a detected ion's peak width as measured by its FWHM or its zero-crossing width in the chromatographic and/or spectral directions also can be determined and stored.

[0298] The zero-crossing width is applicable when filtering is performed with a second derivative filter. The zero value of the second derivative occurs at inflection points of a peak on both the up-slope and down-slope sides of the peak. For a Guassian peak profile, the inflection points occur at ± 1 standard deviation distance from the peak apex. Thus the width as measured by the inflection points correspond to the 2-standard deviation width of the peak. Thus the zero-crossing width is a height-independent measure of peak width corresponding to approximately 2 standard deviations. In an embodiment of the present invention, the number of rows in the table corresponds to the number of ions detected.

[0299] The present invention also provides a data compression benefit. This is because the computer memory needed to store the information contained in the ion parameter table is significantly less than the memory needed to store initially generated original LC/MS data. For example, a typical injection that contains 3600 spectra (for example, spectra collected once per second for an hour), with 400,000 resolution elements in each spectrum (for example, 20,000:1 MS resolution, from 50 to 2,000 amu)

requires in excess of several gigabytes of memory to store the LC/MS data matrix of intensities.

[0300] In a complex sample, using embodiments of the present invention, on the order of 100,000 ions can be detected. These detected ions are represented by a table having 100,000 rows, each row comprising ion parameters corresponding to a detected ion. The amount of computer storage required to store the desired parameters for each detected ion is typically less than 100 megabytes. This storage amount represents only a small fraction of the memory needed to store the initially generated data. The ion parameter data stored in the ion parameter table can be accessed and extracted for further processing. Other methods for storing the data can be employed in embodiments of the present invention.

[0301] Not only are storage requirements significantly reduced, but computational efficiency of post-processing LC/MS data is significantly improved if such analysis is performed using the ion parameter list rather than the originally produced LC/MS data. This is due to the significant reduction in number of data points that need to be processed.

STEP 7: Simplify spectra and chromatograms

[0302] The resulting ion list or table can be interrogated to form novel and useful spectra. For example, as described above, selection of ions from the table based upon the enhanced estimates of retention times produces spectra of greatly reduced complexity. Alternatively, selection of ions from the table based upon the enhanced estimates of m/z values produces chromatograms of greatly reduced complexity. As described in more detail below, for example, a retention time window can be used to exclude ions unrelated to the species of interest. Retention-time selected spectra simplify the interpretation of mass spectra of molecular species, such as proteins, peptides and their fragmentation products, that induce multiple ions in a spectrum. Similarly an m/z window can be defined to distinguish ions having the same or similar m/z values.

[0303] Using the concept of a retention window, simplified spectra from an LC/MS chromatogram can be obtained. The width of the window can be chosen to be no larger than the FWHM of the chromatographic peak. In

some cases, smaller windows such as one tenth the FWHM of a peak are selected. The retention-time window is defined by selecting a particular retention time, which is generally associated with the apex of a peak of interest, and then choosing a range of values about the chosen particular retention time

[0304] For example, the ion having the highest intensity value can be selected and retention time recorded. A retention time window is selected around the recorded retention time. Then, the retention times stored in the ion parameter table are examined. Only those ions having retention times falling within the retention time window are selected for inclusion in the spectrum. For a peak having a FWHM of 30 seconds, a useful value of retention time window can be as large as +/-15 seconds or as small as +/-1.5 seconds.

[0305] The retention time window can be specified to select ions that elute nearly simultaneously, and are thus candidates for being related. Such a retention time window excludes unrelated molecules. Thus, the spectra obtained from the peak list using the retention window contains only the ions corresponding to the species of interest thereby, significantly simplifying the produced spectrum. This is a large improvement over spectra generated by conventional techniques, which typically contain ions unrelated to the species of interest.

[0306] Using the ion parameter table also provides a technique for analyzing chromatographic peak purity. Peak purity refers to whether a peak is due to a single ion or the result of co-eluting ions. For example, by consulting the ion parameter list generated by embodiments of the present invention an analyst can determine how many compounds or ions elute within the time of a principle peak of interest. A method for providing a measure or metric of peak purity is described with reference to Figure 21.

[0307] In step 2102, a retention time window is chosen. The retention time window corresponds to the lift off and touch down of the peak corresponding to the ion of interest. In step 2104, the ion parameter table is interrogated to identify all ions eluting within the chosen retention time window. In step 2106, the intensities of the identified ions (including the

ion of interest) are summed. In step 2108, a peak purity metric is calculated. The peak purity metric can be defined in a number of ways. In one embodiment of the present invention, the peak purity metric is defined as:

$$\text{purity} = 100 * (\text{intensity of peak of interest}) / (\text{sum of intensity of all peaks in retention window}).$$

Alternatively, in another embodiment of the present invention, peak purity is defined as:

$$\text{purity} = 100 * (\text{intensity of most intense}) / (\text{sum of intensity of all peaks in retention window}).$$

In both definitions of peak purity, the peak purity is expressed as a percent value.

[0308] The spectra simplifying properties of the present invention can also be used to study biological samples more easily. Biological samples are an important class of mixtures commonly analyzed using LC/MS methods. Biological samples generally comprise complex molecules. A characteristic of such complex molecules is that a singular molecular species may produce several ions. Peptides occur naturally at different isotopic states. Thus, a peptide that appears at a given charge will appear at several values of m/z , each corresponding to a different isotopic state of that peptide. With sufficient resolution, the mass spectrum of a peptide exhibits a characteristic ion cluster.

[0309] Proteins, which typically have high mass, are ionized into different charge states. Although isotopic variation in proteins may not be resolved by a mass spectrometer, ions that appear in different charge states generally can be resolved. Such ions produce a characteristic pattern that can be used to help identify the protein. The methods of the present invention would then allow one to associate those ions from a common protein because they have a common retention time. These ions then form a simplified spectrum

that can be analyzed by for example, the method disclosed in U.S. Patent No. 5,130,538 to Fenn et al.

[0310] Mass spectrometers measure only the ratio of mass-to-charge, not mass by itself. It is possible however, to infer the charge state of molecules such as peptides and protein from the pattern of ions they produce. Using this inferred charge state, the mass of the molecule can be estimated. For example, if a protein occurs at multiple charge states, then it is possible from the spacing of m/z values to infer the charge, to calculate the mass of each ion knowing the charge and ultimately to estimate the mass of the uncharged parent. Similarly, for peptides, where the m/z charges are due to charge in the isotopic value for a particular mass m , it is possible to infer the charge from the spacing between adjoining ions.

[0311] There are a number of well-known techniques that use the m/z values from ions to infer the charge and parent mass. An exemplary such technique is described in U.S. Patent No. 5,130,538, which is hereby incorporated by reference herein in its entirety. A requirement for each of these techniques is selection of the correct ions and the use of accurate values for m/z . Ions represented in the detected ion parameter table provide high precision values that can be used as inputs to these techniques to produce results with enhanced precision.

[0312] In addition, several of the cited methods attempt to reduce the complexity of spectra by distinguishing between ions that may appear in a spectrum. Generally, these techniques involve selecting a spectrum centered on a prominent peak or combining spectra associated with a single peak, to obtain a single extracted MS spectra. If that peak were from a molecule that produced multiple, time-coincident ions, the spectra would contain all those ions including ions from unrelated species.

[0313] These unrelated species can be from ions that elute at the exact same retention time as the species of interest or, more commonly, the unrelated species are from ions that elute at different retention times. However, if these different retention times are within a window of approximately the FWHM of the chromatographic peak width, the ions from the front or tails of these peaks are likely to appear in the spectrum. The appearance of the

peaks associated with unrelated species requires subsequent processing to detect and remove them. In some instances where they coincide, they may be biasing measurements.

[0314]

Figure 22A illustrates an exemplary LC/MS data matrix that results from two parent molecules, and the resulting multiplicity of ions. In this example a species elutes at time t1 producing 4 ions and another species elutes at time t2 producing 5 ions in the LC/MS data matrix. Even though there are two distinct species, if a spectrum were to be extracted at time t1 or time t2, the result spectrum would contain nine peaks one from each of the nine ions. However the present invention would obtain 9 accurate retention times (as well as m/z and intensities) for each of these 9 ions. If a spectrum were then constructed only of ions that had retention times substantially equal to t1, then only four ions would be present. This simplified spectrum appears in Figure 22B. Similarly, if a spectrum were then constructed only of ions that had retention times substantially equal to t2, then only five ions would be present. This simplified spectrum appears in Figure 22C.

Applications

[0315]

As a sample is collected with an LC/MS system, a plurality of spectra is typically collected across the chromatographic peak in order for the retention time to be accurately inferred. For example, in embodiments of the present invention 5 spectra per FWHM are collected.

[0316]

It is possible to alternate the configuration of an LC/MS system on a spectrum by spectrum basis. For example, all even spectra can be collected in one mode and all odd interleaving spectra can be collected with the MS configured to operate in a different mode. An exemplary dual mode collection operation can be employed in LC/MS alternating with LC/MSE where in one mode (LC/MS) unfragmented ions are collected and in the second mode (LC/MSE), fragments of the unfragmented ions collected in the first mode are collected. The modes are distinguished by the level of a voltage applied to ions as they traverse a collisional cell. In the first mode the voltage is low, and in the second mode, the voltage is elevated.

(Bateman et al.)

[0317]

In such a system, the fragments or ions collected with the system in one mode appear with a chromatographic profile having the same retention time as the unmodified ions. This is because the unfragmented and fragmented ions are common to the same molecular species, so the elution profile of the molecule is imprinted upon all unfragmented and fragmented ions that derive from it. These elution profiles are substantially in time alignment because the extra time required to switch between modes in online MS is short as compared to the peak width or FWHM of a chromatographic peak. For example, the transit time of a molecule in an MS is typically on the order of milli- or micro-seconds, while the width of a chromatographic peak is typically on the order of seconds or minutes. Thus, in particular, the retention times of the unfragmented and fragmented ions are substantially identical. Moreover, the FWHM of the respective peaks will be the same, and further, the chromatographic profiles of the respective peaks will be substantially the same.

[0318]

The spectra collected in the two modes of operation can be divided into two independent data matrices. The operations of convolution, apex detection, parameter estimation and thresholding described above can be applied independently to both. Although such analysis results in two lists of ions, the ions appearing in the lists bear a relationship to one another. For example, an intense ion having a high intensity that appears in the list of ions corresponding to one mode of operation may have counterpart in the list of modified ions collected according to the other mode of operation. In such a case, the ions will typically have a common retention time. To associate such related ions with one another for analysis, a window restricting retention time as described above can be applied to ions found in both data matrices. The result of applying such a window is to identify ions in the two lists having a common retention time and are therefore likely to be related.

[0319]

Even though the retention times of these related ions are identical, the effects of detector noise will result in the measured values of retention time of these ions to differ somewhat. This difference is a manifestation of statistical error and measures the precision of the measurement of retention

time of an ion. In the present invention, the difference in estimate retention times of ions will be less than the FWHM of the chromatographic peak width. For example if the FWHM of a peak is 30 seconds, the variation in retention times between ions will be less than 15 seconds for low-intensity peaks, and less than 1.5 seconds for high-intensity peaks. The window widths used to collect ions of the same molecule (and to reject unrelated ions) can be as large as ± 15 seconds or as small as ± 1.5 seconds in this example.

[0320]

Figure 23 is a graphical chart illustrating how related ions can be identified in the unmodified and modified ion lists generated by an embodiment of the present invention. Data matrix 2302 shows three precursor ions 2304, 2306 and 2308 that are detected in spectra resulting from an unmodified MS experiment. Data matrix 2310 shows eight ions that result from an experiment after the MS is modified for example as described above to cause fragmentation. Ions in data matrix 2302 that are related to those in the data matrix 2310 appear at the same retention time, as indicated by the three vertical lines labeled t_0 , t_1 , and t_2 . For example, ions 2308a and 2308b in data matrix 2310 are related to ion 2308 in data matrix 2302. Ions 2306a, 2306b, and 2306c in data matrix 2310 are related to ion 2306 in data matrix 2302. Ions 2304a, 2304b and 2304c in data matrix 2310 are related to ion 2304 in data matrix 2302. These relationships can be identified by retention time windows with appropriate widths centered at time t_0 , t_1 , and t_2 respectively.

[0321]

The ion parameter list can be used for a variety of analyses. One such analysis involves fingerprinting or mapping. There are numerous examples of mixtures that are, on the whole well characterized, and have essentially the same composition, and whose components exist in the same relative amounts. Biological examples include the end products of metabolism such as urine, cerebrospinal fluid, and tears. Other examples are the protein contents of cell populations found in tissues and blood. Other examples are the enzymatic digests of protein contents of cell populations found in tissues and blood. These digests contain mixtures of peptides that can be analyzed by dual mode LC/MS and LC/MS/MS. Examples

in industry include perfumes, fragrances, flavors, fuel analysis of gasoline or oils. Environmental examples include pesticides, fuel and herbicides, and contamination of water and soil.

[0322] Abnormalities from what would be expected to be observed in these fluids include xenobiotics in the case of products of metabolism that result from ingestion or injection of drugs or drug substances; evidence of drugs of abuse in metabolic fluids; adulteration in products such as juices, flavors, and fragrances; or in fuel analysis. The ion parameter list generated according to embodiments of the present invention can be used as an input to methods known in the art for fingerprint or multi-variate analysis. Software analysis packages such as SIMCA (Umetrics, Sweden), or Pirouette (Infometrix, Woodenville, Washington, USA) can be configured to use fingerprint or multi-variate techniques to detect such abnormalities, by identifying changes in ions between sample populations. These analyses can determine the normal distribution of entities in a mixture, and then identify those samples that deviation from the norm.

[0323] The synthesis of a compound may produce the desired compound together with additional molecular entities. These additional molecular entities characterize the synthetic route. The ion parameter list in effect becomes a fingerprint that can be used to characterize the synthetic route of the synthesis of a compound.

[0324] Another important application to which the present invention is applicable is biomarker discovery. The discovery of molecules whose change in concentration correlates uniquely with a disease condition or with the action of a drug is fundamental to the detection of disease or to the processes of drug discovery. Biomarker molecules can occur in cell populations or in the products of metabolism or in fluids such as blood and serum. Comparison of the ion parameter lists generated for control and disease or dosed states using well known methods can be used to identify molecules that are markers for the disease or for the action of a drug.

[0325] The foregoing disclosure of the preferred embodiments of the present invention has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to

the precise forms disclosed. Many variations and modifications of the embodiments described herein will be apparent to one of ordinary skill in the art in light of the above disclosure. The scope of the invention is to be defined only by the claims appended hereto, and by their equivalents.

[0326]

Further, in describing representative embodiments of the present invention, the specification may have presented the method and/or process of the present invention as a particular sequence of steps. However, to the extent that the method or process does not rely on the particular order of steps set forth herein, the method or process should not be limited to the particular sequence of steps described. As one of ordinary skill in the art would appreciate, other sequences of steps may be possible. Therefore, the particular order of the steps set forth in the specification should not be construed as limitations on the claims. In addition, the claims directed to the method and/or process of the present invention should not be limited to the performance of their steps in the order written, and one skilled in the art can readily appreciate that the sequences may be varied and still remain within the spirit and scope of the present invention.